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THE BROMINATION OF FURYL METHYL KETONE

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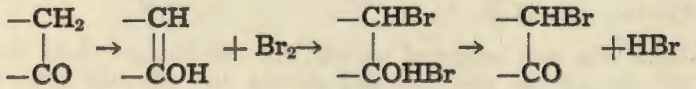
The great tendency of furan to undergo nuclear substitution when treated with a reagent which can effect furan nuclear substitution has been demonstrated in the case of ethyl furylacrylate[1] and 2-furyl phenyl ketone[2]. The only exception has been the addition of bromine to the side chain of furyl ethylene[3]. In the bromination of furyl methyl ketone it has been shown that the bromine enters the side chain to give *w*-bromofuryl methyl ketone. This behavior may be explained by the theory of C. F. Ward[4]. This theory assumes enolization of the carbonyl group and addition to the unsaturated linkage with a final removal of hydrogen bromide.



It was expected, however, that the second atom of bromine would substitute in the nucleus. This was shown not to be the case as the dibromination of furyl methyl ketone gives *w,w*-dibromofuryl methyl ketone in 90 per cent yield. It was thought that this formation of a chain substituted compound was caused by the removal of hydrogen bromide from an addition product which may have contained bromine attached to the nucleus. To test this theory the bromination was carried out at low temperature and the product treated with alcoholic potassium hydroxide before it reached a point where hydrogen bromide was given off spontaneously. However, a careful examination of the reaction product failed to show a compound containing a bromine atom in the nucleus.

The nitration product of furyl methyl ketone[5] has been shown to be 5-nitro-2-furyl methyl ketone by comparison with the product from the reaction of diazomethane with 5-nitro-2-furfural[6]. This ketone has been characterized by the preparation of the oxime.

EXPERIMENTAL

MONOBROMINATION OF FURYL METHYL KETONE

To 11 g. (0.8 mole) of furyl methyl ketone dissolved in 100 cc. of carbon disulfide (dried over calcium chloride), 16 g. (0.1 mole) of bromine

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[1] Gilman and Wright, *J. Am. Chem. Soc.*, 52, 3349 (1930).
[2] Gilman and Young, *J. Am. Chem. Soc.*, 56, 464 (1934).
[3] Moureu, Dufraisse and Johnson, *Ann. chim.*, 7, 8 (1927).
[4] C. F. Ward, *J. Chem. Soc.*, 123, 2207 (1923).
[5] Rinkes, *Rec., trav. chim.*, 51, 349 (1932).
[6] Gilman and Wright, *J. Am. Chem. Soc.*, 52, 2250 (1930).

in 500 cc. of carbon disulfide was added. This bromine was added dropwise with stirring at room temperature. The mixture was stirred 15 minutes after the last addition of bromine. It was then poured into water and washed with sodium bicarbonate solution. The carbon disulfide layer was dried and most of the carbon disulfide removed. The residue was distilled under reduced pressure. The fraction boiling 120-5° C. at 20 mm. pressure was collected and redistilled. The yield of redistilled *w*-bromofuryl methyl ketone b.p. 121-3° C. at 20 mm. pressure was 16 g. This was 90 per cent of the theoretical amount. The product thus obtained was a liquid and would not solidify on cooling. Other constants are:

N_D^{25} 1.5783; D_4^{25} 1.5785.

MR_D: Calcd 36.57, Obs. 39.75

However, if this oil were dissolved in petroleum ether at room temperature and then vigorously cooled, crystals were deposited which after two crystallizations melted constantly at 36-7° C.

Anal. Calcd. for $C_6H_5O_2Br$: Br, 42.33
Found: Br, 42.26. 42.21

The above run was repeated except that the temperature was kept below — 15° during the reaction and during the stirring after the reaction. No hydrogen bromide was evolved. A portion of the product was treated with alcoholic sodium hydroxide at 0° C. Another portion was treated with pyridine and the rest was allowed to come up to room temperature and evolve hydrogen bromide. From none of these fractions was any compound isolated which contained nuclear bromine.

OXIDATION OF w-BROMOFURYL METHYL KETONE

Two grams (0.01 mole) of *w*-bromofuryl methyl ketone and 4 g. of calcium hydroxide were suspended in ice water and 1 g. of potassium permanganate in water was added. The mixture was heated to boiling and filtered. The solution was then acidified and extracted with ether, yielding 0.4 g. of furoic acid. This was 35 per cent of the theoretical amount. The furoic acid was identified by its melting point of 129-30° C. and a mixed melting point with an authentic sample showed no depression.

Three grams (0.015 mole) of *w*-bromofuryl methyl ketone were treated with 3 cc. of pyridine in 50 cc. of dry ether and refluxed one hour. The reaction mixture was cooled and a layer of gummy material separated. A portion of this gummy material which was undoubtedly furacyl pyridinium bromide was then dissolved in water and treated with sodium hydroxide at 40°. The solution was then acidified and extracted with ether, giving furoic acid. The furoic acid was identified by its melting point, 129-130° C. and by mixed melting point with an authentic sample.

w-BROMOFURYL METHYL KETONE FROM THE FRIEDEL-CRAFTS REACTION

To 30 g. (0.24 mole) of aluminum chloride in 50 cc. of carbon disulfide, 41 g. (0.2 mole) of bromacetyl bromide in 100 cc. of carbon disulfide were added at room temperature. Fourteen grams (0.2 mole) of furan in 50 cc. of carbon disulfide were added to this mixture. It was stirred

for 15 minutes after the addition and then poured into ice-water to decompose it. The carbon disulfide layer was washed several times with sodium bicarbonate solution, dried, and the solvent was removed. The residue was distilled and the fraction boiling at 122-5° C. at 20 mm. was collected. The yield of *w*-bromofuryl methyl ketone was 7 g. This was 38 per cent of the theoretical amount. The oily product was crystallized from petroleum ether. M.P. 36-37° C. It was shown to be the same compound as that derived from the bromination of furyl methyl ketone as there was no depression of the melting point when the two were mixed.

DIBROMINATION OF FURYL METHYL KETONE

Thirty-two grams (0.2 mole) of bromine in 50 cc. of carbon disulfide were added dropwise with stirring at room temperature to 11 g. (0.1 mole) of furyl methyl ketone. When the hydrogen bromide evolution ceased the reaction mixture was poured into water, washed with sodium bicarbonate solution, dried, and the solvent removed. The residue was distilled under reduced pressure. The fraction boiling at 140-150° C. at 15 mm. pressure was collected. On refractionation the fraction boiling at 145-147° C. at 15 mm. pressure was collected. The yield of *w,w'*-dibromofuryl methyl ketone was 24.5 g. This was 90 per cent of the theoretical amount. Other constants are:

N_D^{25} 1.6070. D_4^{25} 2.0040
M.R. Calcd. 76.2, Obs. 81.2
Anal. Calcd. for $C_6H_4O_2Br_2$: Br, 59.70
Found: Br, 59.30, 59.37.

OXIDATION OF w,w'-DIBROMOFURYL METHYL KETONE

Five grams of the ketone were refluxed in ether with 5 cc. of pyridine. No salt separated on cooling so the ether solution was extracted with water and the water extract treated with sodium hydroxide at 40° C. The solution was acidified and extracted with ether, giving 0.5 g. of furoic acid m.p. 129-130° C. This was 20 per cent of the theoretical amount. The acid was identified by a mixed melting point with an authentic sample and showed no halogen test on fusion with sodium.

Five grams (0.02 mole) of the ketone and 10 g. of calcium hydroxide were suspended in ice water and treated with 2.5 g. of potassium permanganate in solution. The mixture was heated, acidified and extracted with ether, giving 1.4 g. of acid. This was 65 per cent of the theoretical amount of furoic acid melting point 125-127° C. The furoic acid was identified by mixed melting point with an authentic sample and showed no halogen test on fusion with sodium.

All the other fractions from a dibromination run were submitted to oxidation in the above manner but no trace of a compound containing nuclear halogen was found.

BROMINATION OF 5-BROMOFURYL METHYL KETONE

Ethyl 5-bromofuroate was converted to ethyl 5-bromofuroyl acetate by the Claissen condensation in 34 per cent yield. The ethyl 5-bromofuroyl acetate was hydrolyzed by dilute sulfuric acid in 80 per cent yield,

giving 5-bromofuryl methyl ketone. To 30 g. (0.16 mole) of 5-bromofuryl methyl ketone in 100 cc. of carbon disulfide, 24 g. (0.15 mole) of bromine were added at room temperature with stirring. The mixture was stirred until the hydrogen bromide evolution ceased and it was then poured into water. The carbon disulfide was washed with sodium bicarbonate solution, dried, and the solvent removed. The residue was distilled under reduced pressure. The fraction boiling 150-155° C. at 19 mm. pressure was collected. It solidified in the receiver and was then recrystallized to the constant melting point of 98.5-99.5° C. The yield was 21 g. of *w*,5-dibromofuryl methyl ketone. This was 50 per cent of the theoretical amount.

Anal. Calcd. for $C_6H_4O_2Br_2$: Br, 59.70
Found: Br, 59.91, 59.85.

OXIDATION OF w, 5-DIBROMOFUYRL METHYL KETONE

To 1 g. (0.004 mole) of the ketone and 2 g. of calcium hydroxide suspended in ice water 0.6 g. of potassium permanganate was added in solution. The reaction mixture was heated, acidified, and extracted with ether, giving 0.25 g. of 5-bromofuroic acid. This was 36 per cent of the theoretical amount. The acid on recrystallization melted at 184° C. and a mixed melting point with an authentic sample showed no depression.

To 1 g. (0.004 mole) of the ketone 1 cc. of pyridine in ether was added. The mixture was refluxed one hour and filtered. The residue was dissolved in water, treated with sodium hydroxide at 40° C., acidified, and extracted with ether, yielding 0.5 g. of acid. This was 70 per cent of the theoretical amount of 5-bromofuroic acid. This acid was identified by its melting point and by a mixed melting point with an authentic sample.

5-NITROFURYL METHYL KETONE

5-Nitrofuryl methyl ketone was prepared by the method of Rinkes. This compound was shown to be 5-nitro-2-acetylfuran by the following reaction. Diazomethane in ether was prepared by heating 45 g. (0.8 mole) of potassium hydroxide in 200 cc. of methyl alcohol with 25 g. (0.2 mole) of nitrosomethyl urethane in 300 cc. of ethyl ether and distilling the mixture of diazomethane and ether. To this solution 14.1 g. (0.1 mole) of 5-nitrofurfural were added. A rapid evolution of nitrogen took place and, after the completion of the reaction, the ether was removed and the product crystallized. The melting point of 5-nitro-2-acetylfuran was 78-78.5° C. When mixed with the nitrofuryl methyl ketone of Rinkes it was 78-78.5° C.

5-NITROFURYL METHYL KETOXIME

Because of the sensitivity of the furan nitro group to alkali, this oxime was prepared in acid solution. To 5 g. (0.03 mole) of 5-nitrofuryl methyl ketone and 4 g. (0.06 mole) of hydroxylamine hydrochloride in alcohol solution one-half cc. of concentrated hydrochloric acid was added. The mixture was heated to 100° C. for 2 hours in a closed vessel. The yield of 5-nitrofuryl methyl ketone, melting point 167-168° C., was 5.1 g. This was 93 per cent of the theoretical amount.

SUMMARY

1. It has been shown that in the bromination of 2-furyl methyl ketone the first bromine enters the side chain. It has been further shown that the second bromine will also enter the side chain.

2. In the bromination of 5-bromo-2-furyl methyl ketone the bromine enters the side chain.

3. The nitrofuryl methyl ketone prepared by Rinkes has been shown to be 5-nitro-2-furyl methyl ketone.

THE OXIDATION OF FURAN METHYL GROUPS

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Although oxidation in the furan series has been studied since 1873, when Limpricht[1] worked with furoic acid, no general method and few special methods have been reported for the oxidation of furan compounds without decomposition of the furan ring. Some of the agents that have been used for the oxidation of furan compounds to alipathic acids such as fumaric, succinic, oxalic, etc., are: aqueous bromine[2], dilute nitric acid[3], potassium permanganate[4], peracetic acid[5], sodium chlorate and vanadium pentoxide or osmium tetroxide[6], air and vanadium trioxide, venadium pentoxide, or ammonium vanadate[7], oxygen and vanadium pentoxide[8], Caro's acid[9], and many others. There are a number of isolated cases of oxidation in which the furan ring is kept intact, but only a few will be enumerated. Boeseken[10] and his coworkers using 70 per cent peracetic acid in acetic acid solution, have obtained what they consider to be an oxide of furan. Furfural and other furan aldehydes have been oxidized to the corresponding acids by potassium permanganate[11], silver oxide[2], air[12], and other agents. The Cannizzaro reaction goes smoothly with a number of furan aldehydes. Furoin has been oxidized to furil by air[13] or electrolysis[14]. Moureu, Dufraisse, and Johnson[15] have reported the supposed oxidation of furylbromoethylene with air to *w*-bromofuryl methyl ketone. Priebs[16] has oxidized 5-nitro-2-ethenyl nitrofuran to 5-nitro-2-furoic acid with chromic acid. Gilman and Wright[17] have oxidized 5-nitro-furfuryl alcohol to nitrofurfural by heating to 40-50° for two days with manganese dioxide and 50 per cent sulfuric acid.

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[1] Limpricht, *J. Chem. Soc.*, 26, 624 (1873).
[2] Hill and Jennings, *Proc. Am. Acad.*, 27, 186 (1891).
[3] Hill and Hartshorn, *Ber.* 18, 448 (1885).
[4] Atterberg, *J. Chem. Soc.*, 40, 663 (1881).
[5] Boeseken and coworkers, *J. Chem. Soc.*, (T) 75, 747 (1899).
[6] Milas, *J. Am. Chem. Soc.*, 49, 2005 (1927).
[7] Boehringer, *British Pat.*, 297667.
[8] Sessions, *J. Am. Chem. Soc.*, 50, 1696 (1928).
[9] Cross and coworkers, *Ber.*, 31, 43 (1898); *Chem. News*, 82, 1631 (1900).
[10] Boeseken and coworkers, *Rec. trav. chim.*, 50, 1023 (1931).
[11] (a) Volhard, *J. Chem. Soc.*, 60, 896 (1891); *Ann.*, 261, 379 (1895).
(b) Gilman and Wright, *Rec. trav. chim.*, 50, 833 (1931).
[12] Moureu, Defraisse, and Lotte, *Comp. rend.*, 180, 993 (1925).
[13] E. Fisher, *J. Chem. Soc.*, 40, 798 (1880).
[14] Law, *J. Chem. Soc.*, 89, 1445 (1906).
[15] Moureu, Dufraisse, and Johnson, *Ann. Chim. phys.*, 7, 14 (1927).
[16] Priebs, *Ber.*, 18, 1362 (1885).
[17] Gilman and Wright, *J. Am. Chem. Soc.*, 53, 1923 (1931).

Methyl groups in the furan series have been oxidized[18] by bromination to give the dibromide followed by hydrolysis to the aldehyde. The drastic conditions of this method, however, keep it from being general.

The oxidizing agent used in these studies is one that has been applied successfully by W. A. Noyes[19] to the benzene series, and appears to be general for the oxidation of furan methyl groups to carboxylic acids. This reagent, potassium ferricyanide, combines power with mildness in a way which makes it an ideal oxidizing agent in the furan series. With it such compounds as furfuryl alcohol and sylvan have been oxidized to furoic acid.

EXPERIMENTAL

TYPICAL OXIDATION FOR MOST TYPES OF FURAN COMPOUNDS

Oxidation in basic solution may be used for all compounds except the nitrofurans which are unstable in the presence of alkalies. A typical oxidation is outlined.

One gram of the compound, 25 g. of potassium ferricyanide, and 10 g. of potassium hydroxide were placed in a 300 cc. flask with 150 cc. of distilled water and refluxed three hours. The solution was filtered hot to remove iron oxide, and concentrated to one-half the original volume. On cooling a large quantity of potassium ferrocyanide crystallized and was filtered. This potassium ferrocyanide could be converted to potassium ferricyanide and used again. The filtrate was then acidified with hydrochloric acid in slight excess of that required to neutralize the base. It was not necessary to use enough hydrochloric acid to convert the potassium ferrocyanide to the acid as hydroferrocyanic acid was stronger than the organic acids prepared. The acid solution was extracted with ether. Removal of the ether left the impure organic acid which was purified by crystallization. In some cases where the yields were poor it was found advantageous after boiling two hours to add another 25 g. of potassium ferricyanide and 10 g. of potassium hydroxide and continue the boiling another two hours. Table 1 gives the amounts of reagents used and the yields of acid in grams.

OXIDATION OF GROUPS IN FURAN NITRO COMPOUNDS

In the case of the furan nitro compounds, two equivalents, 34 g. of potassium acetate, were used instead of the 10 g. of potassium hydroxide. The procedure was the same as before with this exception. The neutral type of reaction, using potassium acetate, was also tried with some of the other furan compounds.

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[18] Hill and Sawyer, *Am. Chem. J.*, **20**, 169 (1898).

[19] (a) W. A. Noyes, *Am. Chem. J.*, **5**, 97 (1883); (b) **7**, 145 (1885); **8**, 176 (1886); (d) **9**, 93 (1887); (e) **10**, 472 (1888); (f) **11**, 161 (1889).

TABLE 1.

Compounds oxidized	Sample grams	Grams potassium ferricyanide used	Acid obtained	Yield grams
Sylvan	1	50	furoic	.05
Dimethyl furan	1	25	dehydromucic	.01
Furyl methyl ketone	1	25	furoic	.51
Furylacrylic acid	1	25	not oxidized	
5-Methyl-2-furoic	1	25	dehydromucic	.35
Furfural	1	25	furoic	.22
5-Bromofuryl methyl ketone	1	25	5-bromofuroic	.45
Furyl methyl ketone[1]	1	25	furoic	.30
5-Nitrosylvan[1]	1	25	5-nitrofuroic	.54
Furfuryl alcohol	1	25	furoic	.21
Furfural acetone	1	25	furylacrylic	.10
Furfural acetone[1]	1	25	furylacrylic	.10
Furil	½	25	furoic	.41
2-Methyl-3-furoic acid	1	75	2,3-dicarboxyfuran	.35
Tertiarybutyl furoic acid	½	50	dehydromucic	.01
Furyl ethylene	1	25	furoic acid	.02

[1] All of these were run in neutral solution.

The acids were characterized by m.p. and mixed m.p. with authentic samples.

In the case of dehydromucic acid, which has no melting point, the acid was converted to the dimethyl ester and a m.p. and a mixed m.p. were taken.

SUMMARY

1. An oxidizing agent which seems to be general for the oxidation of furan methyl groups has been reported. A number of other furan compounds have been oxidized using the same reagent.

THE AVAILABILITY OF PHOSPHORUS IN SOME IOWA SOILS[1]

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The phosphorus content of most Iowa soils, according to the results of analyses of all types mapped in the state, is quite low. The amount present has actually been found to vary from as low as 0.015 per cent up to 0.12 per cent, the average figure for 1,345 analyses of samples of the various types being 0.0657 per cent. But the total amount of phosphorus present in the soil often bears no relation to the amount available for plant use. Hence, much importance is attached to the problem of the determination of the available phosphorus content of soils and its relationship to the total supply. Many methods have been proposed for the estimation of the available phosphorus in soils but none has yet been devised which measures accurately the amount present in all soils. The best method now available is still the laborious and time-consuming determination of crop responses to phosphate fertilizers in field experiments.

The object of the present investigation was to determine the available phosphorus content of a number of Iowa soils using various methods and to compare the results with the crop response to phosphate fertilizers in field tests. It was also hoped that the data would throw some light on the problem of the factors influencing availability.

The 1 per cent citric acid method proposed by Dyer (2) in 1894 marks the first important use of a weak acid solvent for determining the available phosphorus in soils. Since the work of Dyer many other methods using various dilute acids have been proposed (1), (5), (16), (21).

Von Wrangell (22) and McGeorge (8) have studied the rate of solution of phosphorus in the soil, the former by water extraction and the latter by electrodialysis, in an attempt to determine the availability of the phosphorus. Hibbard (5) suggested a modification of the von Wrangell procedure and Fisher and Thomas (3) proposed the use of two solvents to differentiate various forms of phosphorus in the soil.

Truffaut and Bezssonoff (20) advocated the use of a mixed culture of *Clostridium pasteurianum, Bacillus truffauti* and *Azotobacter agile* to measure available phosphorus. This method is based on the assumption that since bacteria are plants and require phosphorus for their normal growth and development, their requirements will represent those of higher plants. Certain species of these bacteria living in the soil in the presence of an abundant supply of phosphorus are able to decompose sugar and secure from the air the nitrogen which they need for growth. The amount of nitrogen fixed by these bacteria is proportional to the growth they make and the growth made is proportional to the amount of phosphorus the bacteria are able to secure from the soil. The amount of nitrogen fixed by a culture of these bacteria in a medium with the soil in question as the source

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[1] Journal Paper No. J438 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 228.

of phosphorus is regarded, therefore, as a measure of the available phosphorus.

Niklas et al (11) found that *Aspergillus niger* made more growth in a solution well supplied with phosphorus than in a solution poor in this element. They claim that the amount of growth made in a soil suspension by this mold is proportional to the amount of available phosphorus in that soil.

Another test (9) similar to the *A. niger* method depends upon the development of a mold of the genus Cunninghamella when seeded on the surface of the soil in small glass dishes. The assumption is also made in this test that the development of the mold is proportional to the available phosphorus present in the soil.

Neubauer (10) proposed the seedling plant method for determining the available phosphorus in the soil. This method consists of planting 100 rye seeds in a small glass dish of the soil to be tested and with proper temperature and moisture conditions it is claimed the rye seedings will grow until they exhaust the food supply of the soil. The seedlings are then analyzed for phosphorus and the amount found is supposed to represent the amount of available phosphorus in the soil. Both favorable (19) and unfavorable (13) results have been reported from the use of this method.

These methods are all based upon certain assumptions and this brief review shows that not all of the assumptions made are valid. However, a certain degree of success has been attained by the use of the various methods and certainly much information concerning the factors affecting phosphorus availability has been secured.

METHODS OF PROCEDURE

1. *Cooperative Soil Experiment Fields*

Samples of soils were collected in the fall of 1933 from the untreated plots, the limed plots and the lime plus rock phosphate treated plots from 8 of the cooperative soil experiment fields and from the untreated and the rock phosphate treated plots of the Everly field experiment (table 1). The crop yields obtained on all these plots from 1924-1933 were averaged and are shown in table 2. The soils were brought into the laboratory, air-dried and passed through the 2 mm. sieve. The air-dry soils were thoroughly mixed and small representative samples were ground to pass the 100-mesh sieve for the analyses.

The pH of the unground soils was determined on the air-dry samples by the quinhydrone electrode. The lime requirement was determined on the ground sample according to the procedure recommended by Hardy and Lewis (4). The total phosphorus content of the soils was determined by the magnesium nitrate method. The results of these tests are shown in table 3.

2. *Biological Methods*

The availability of the phosphorus in these soils was determined by four biological methods, viz.: the bacteriological method of Truffaut and Bezssonoff (20), the *A. niger* method of Niklas et al (11), the Cunninghamella method (9), and the Neubauer method (10). In the use of the bacteriological method certain modifications were necessary. *B. truf-*

TABLE 1. *Outline of field soil treatment*

Soil No.	Soil type	Field	Treatment*
1	Carrington loam	Waverly	O
2	" "	"	L
3	" "	"	LRP
4	Carrington silt loam	Springville	O
5	" " "	"	L
6	" " "	"	LRP
7	Grundy silt loam	Agency	O
9	" " "	"	L
10	" " "	"	LRP
11	Grundy silty clay loam	West Union	O
13	" " " "	" "	L
14	" " " "	" "	LRP
15	Lamoure silty clay loam	Everly	O
16	" " " "	"	RP
17	Marshall silt loam	Red Oak	O
19	" " "	" "	L
20	" " "	" "	LRP
21	Muscatine silt loam	Letts	O
23	" " "	"	L
24	" " "	"	LRP
25	O'Neill loam	Everly	O
26	" "	"	L
27	" "	"	LRP
28	Tama silt loam	Hudson	O
29	" " "	"	L
30	" " "	"	LRP

* O = No treatment
L = Lime
LRP = Lime + rock phosphate

fauti was not available and this organism was eliminated from the culture. *Azotobacter chroococcum* was used instead of *Az. agile.*

3. *Chemical Methods*

These methods are based upon the assumption that the reagents used will dissolve out only the phosphorus available to the plants and no more. A number of weak acids or dilute solutions of strong acids have been used for this purpose. Most of the chemical methods involve the use of the Denigés colorimetric method for the determination of the phosphorus The differences in the methods are, for the most part, differences in the manner of extraction and in the extracting solution. In this work the Bray (1) test using hydrochloric acid, the Truog (21) test using 0.002N sulfuric acid, the Dyer (2) 1 per cent citric acid method and 1 per cent acetic acid were employed. In addition to these solutions hydrochloric acid solutions of 0.05 and 0.005 N concentrations were used. A saturated solution of carbon dioxide at 25° C. and 50 mm. of water pressure was used, Fig. 1. The procedure followed in the determinations by hydrochloric and carbonic acid was the same as that followed in the Truog method except that these acids were used instead of sulfuric acid. Calcium

TABLE 2. *Average yields of crops on outlying field experiments*
(10-year average, 1924-1933, inclusive)

Soil No	Soil Type	Treatment	Corn Bu/A	Oats Bu/A	Winter Wheat Bu/A	Soy beans Bu/A	Soy beans tons/A	Clover, mixed clover hay tons/A	Alfalfa tons/A
1	Carrington loam	O	39.3[4]**	38.5[3]			0.42[2]		
2	" "	L	51.3	47.6			1.53		
3	" "	LRP	51.8	59.1			1.81		
4	Carrington silt loam	O	49.3[4]	42.9[4]			0.73		
5	" " "	L	48.2	47.7			1.05		
6	" " "	LRP	43.3	56.2			1.41		
7	Grundy silt loam	O	61.4[4]	61.5[3]	20.0[2]		1.59[2]		
9	" " "	L	68.1	65.8	23.5		1.92		
10	" " "	LRP	74.1	72.4	28.5		2.23		
11	Grundy silty clay loam	O	58.5[6]	39.2	16.7	20.3			
13	" " " "	L	58.1	30.9	14.0	19.2			
14	" " " "	LRP	59.2	39.6	16.0	18.9			
15	Lamoure silty clay loam	O	52.1[4]	53.4					1.86[4]
16	" " " "	RP	55.9	72.4					2.71
17	Marshall silt loam	O	65.0[2]		8.6	9.9			3.39[4]
19	" " "	L	66.5		10.2	13.2			3.56
20	" " "	LRP	62.9		13.0	12.3			3.71
21	Muscatine silt loam	O	66.7[5]	57.4[2]	19.1*			0.50	
23	" " "	L	64.1	59.1	20.1*			0.75	
24	" " "	LRP	74.9	60.4	26.3*			1.32	
25	O'Neill loam	O	40.9[5]	44.0[3]				0.43	1.41[2]
26	" "	L	40.6	52.0				0.36	1.40
27	" "	LRP	50.0	49.4				0.41	1.53
28	Tama silt loam	O	48.1[5]	46.9[3]				1.69	
29	" " "	L	56.7	53.4				2.27	
30	" " "	LRP	59.8	60.7				2.32	

* Barley.

** Numbers above and to the right of the figure refer to number of crops.

TABLE 3. *Reaction, lime requirement and total phosphorus content of soils*

Soil No.	Treatment	pH	Lime requirement tons per acre Hardy-Lewis method	Pctg. total phosphorus (oven-dry soil)
1	O	5.40	2.5	0.062
2	L	6.28	0.8	0.060
3	LRP	6.90	0.7	0.103
4	O	5.34	4.7	0.060
5	L	6.78	1.2	0.056
6	LRP	6.66	1.1	0.080
7	O	5.26	4.5	0.056
9	L	4.93	2.7	0.062
10	LRP	5.72	1.5	0.090
11	O	6.36	1.4	0.077
13	L	6.13	1.0	0.066
14	LRP	5.78	1.0	0.103
15	O	8.22	....	0.070
16	RP	8.24	....	0.109
17	O	5.84	2.2	0.070
19	L	6.40	1.0	0.064
20	LRP	6.48	1.0	0.079
21	O	5.10	4.6	0.064
23	L	6.04	1.3	0.060
24	LRP	5.73	1.4	0.071
25	O	6.06	1.9	0.065
26	L	6.65	0.8	0.068
27	LRP	6.98	1.0	0.073
28	O	5.36	4.0	0.054
29	L	6.88	1.1	0.053
30	LRP	6.90	1.0	0.078

and iron were determined in the extracts of 0.05 and 0.005 N hydrochloric acid.

4. *Effect of Lime and Aluminum Sulfate on the pH and Availability of Phosphorus in the Soil*

Each of the 30 soils was treated in duplicate in 3 series as follows:

Series 1—Check

Series 2—$CaCO_3$ in the amount of 4 times the lime requirement

Series 3—1 per cent aluminum sulfate

The soils were treated in tumblers, the moisture was adjusted to 25 per cent and maintained at this amount by frequent additions of distilled water. After 51 days the soils were air-dried and the pH and phosphorus soluble in 0.002 N H_2SO_4 determined.

RESULTS

The average crop response to phosphorus additions varied on the different soils with the crop grown, as appears in table 2. For example, corn did not show any significant response to phosphorus on the Carrington loam and the Carrington silt loam, but with oats and soybeans there was considerable response to phosphorus on these soils. On the other

TABLE 4. *Available phosphorus by biological methods*

Soil No.	Treatment	p.p.m. available P bacteriological method	Mgm. *A. niger* mycelium	p.p.m. available P Cunningham-ella method	Mgm. P_2O_5 Neubauer method
1	O	19.0	90.2	12.5	2.5
2	L	.....	116.9	1.5	1.7
3	LRP	76.5	250.3	trace	7.1
4	O	.....	80.1	11.5	0
5	L	.....	75.0	3.5	3.3
6	LRP	.....	233.6	5.5	1.1
7	O	55.5	66.3	45.0	5.0
9	L	.....	53.9	91.0	.....
10	LRP	189.0	173.5	100.0	.....
11	O	17.0	64.7	100.0	4.1
13	L	.....	82.8	100.0	6.2
14	LRP	188.5	181.5	100.0	5.2
15	O	225.5	126.9	trace	0.6
16	RP	52.0	123.1	.....	6.6
17	O	21.0	103.7	17.5	1.9
19	L	.....	111.6	6.5	.....
20	LRP	333.5	190.6	20.0	.....
21	O	177.5	88.2	20.0	6.9
23	L	227.5	120.4	trace	.....
24	LRP	161.5	161.6	.....	8.5
25	O	160.5	119.7	.....	.....
26	L	108.5	130.5	trace	23.5
27	LRP	129.5	180.3	trace	19.7
28	O	169.5	82.3	15.0	.....
29	L	237.5	80.7	.....	.....
30	LRP	70.0	153.2	trace	.....

hand, corn showed a marked response to phosphorus on the Muscatine silt loam and the O'Neill loam, but with oats there was little or no effect of the phosphorus. No better explanation of these data apparently can be offered than the fact that the phosphorus exists in these soils in different forms and the different crops vary in their ability to assimilate the phosphorus from the existing forms. It is possible that nitrogen or potassium may be the limiting factor for corn on the Carrington loam but this does not seem probable in view of the yields of oats and other crops on this soil.

The reaction of all the untreated soils was acid, except the Lamoure silty clay loam. The pH of all the untreated soils was below 6.0, except with the O'Neill loam in which the pH was 6.06. The pH of the limed soils varied from 4.93 in the Grundy silt loam to 6.90 in the Tama silt loam. The total phosphorus content varied from 0.053 per cent in the limed Tama silt loam to 0.109 per cent in the phosphate treated Lamoure silty clay loam. The reaction and total phosphorus in the soils 4, 5 and 6, from the Springville field, and soils 28, 29 and 30 from the Hudson field, respectively, were quite similar.

The data in table 4 indicate considerable difference in the amount of available phosphorus in the different soils. However, there was no relation between the amount of available phosphorus in any one soil as meas-

ured by the various methods. According to the bacteriological method, the Marshall silt loam contained the largest amount of available phosphorus but this soil gave no response to phosphate fertilizer on corn and only small increases with other crops, such as wheat, soybeans and alfalfa. By the *A. niger* test this soil was third highest in available phosphorus and contained considerably more available phosphorus than the same soil untreated or that treated with lime. According to the Cunninghamella and Neubauer methods the Marshall silt loam contained only a small amount of available phosphorus. In general, the soils treated with phosphate fertilizers contained larger amounts of available phosphorus than the same soils untreated or those treated with lime. However, this was not true for all soils nor was it shown consistently by any one method.

The data in table 5 show that not one of the five chemical methods employed indicated accurately the phosphorus needs of the soil as measured by crop response to phosphorus fertilizers. The simple qualitative test employing HCl was probably the best indicator for all soils and the carbonic acid extraction was probably the most consistent quantitative

TABLE 5. *Available phosphorus by chemical methods*

Soil No.	Treatment	HCl* (Bray)	0.002 N H_2SO_4	1% citric acid	1% acetic acid	H_2CO_3
			p.p.m.	p.p.m.	p.p.m.	p.p.m.
1	O	+	5.21	207.0	1.59	.....
2	L	+++	5.92	238.0	3.18	22.8
3	LRP	+++	199.80	.....	87.40	55.6
4	O	+	5.38	92.0	1.09	18.1
5	L	+++	5.47	25.0	3.84	10.0
6	LRP	++++	118.90	258.0	77.20	52.0
7	O	+	7.59	.....	2.71	21.6
9	L	+++	11.12	.....	4.51	25.3
10	LRP	++++	66.80	212.0	121.30	56.5
11	O	++++	82.99	115.0	71.30	16.7
13	L	++++	40.14	92.0	81.30	16.7
14	LRP	++++	24.75	402.0	83.50	45.7
15	O	++++	24.75	51.0	47.50	37.3
16	RP	++++	25.45	554.0	65.80	23.4
17	O	++++	37.96	11.5	29.00	33.1
19	L	++++	27.10	40.2	22.10	28.4
20	LRP	++++	114.69	157.0	86.60	37.1
21	O	++	13.73	224.0	5.88	28.5
23	L	+++	32.42	379.0	27.90	22.4
24	LRP	++++	120.36	232.0	85.80	56.2
25	O	+++	17.03	299.0	17.50	.....
26	L	+++	9.21	115.0	3.20	11.7
27	LRP	++++	72.21	350.0	42.50	14.6
28	O	+	10.46	117.0	1.11	20.3
29	L	+++	8.55	103.0	1.89	23.5
30	LRP	++++	69.11	.....	35.30	30.9

* + Doubtful
++ Low
+++ Medium
++++ High

TABLE 6. *Phosphorus, calcium and iron soluble in 0.05 N HCl*

Soil No.	Treatment	P in p.p.m.	Ca in p.p.m.	Fe in p.p.m.
1	O	21.2	3689	927
2	L	24.1	4918	1261
3	LRP	273.7	8607	1341
4	O	13.1	4099	1501
5	L	13.6	4099	1033
6	LRP	208.0	6558	1466
7	O	14.6	3689	650
9	L	14.7	4099	485
10	LRP	197.4	6558	700
11	O	183.5	10247	342
13	L	135.6	9427	420
14	LRP	274.7	9427	516
15	O	144.3	13116	1017
16	RP	312.0	20495	600
17	O	91.7	6558	630
19	L	64.1	6968	300
20	LRP	148.5	6968	49
21	O	16.3	4508	548
23	L	46.4	6968	346
24	LRP	104.0	4918	412
25	O	42.3	2869	864
26	L	29.3	5738	1001
27	LRP	152.9	7788	1168
28	O	11.1	3279	864
29	L	14.5	5738	941
30	LRP	114.2	7378	1190

measure in soils below pH 7.0. The variations in amounts of phosphorus soluble by this method appeared to be related to soil differences and especially to the pH of the soil and the presence of rock phosphate. The 1 per cent citric acid extraction gave higher results than the 0.002 N sulfuric acid in every case, except with soil No. 17. This was probably caused by a precipitation of the iron by the citric acid. The results obtained with the 1 per cent acetic acid extraction were similar to those with the 0.002 N sulfuric acid but somewhat lower in most soils.

The phosphorus, calcium and iron soluble in 0.05 N and 0.005 N HCl were determined in each of the soils. The data in tables 6 and 7 show that with the more concentrated HCl solutions the amount of soluble phosphorus was increased. The soils that contained large amounts of calcium were the soils that failed to show large amounts of soluble phosphorus with solvents other than HCl. The soluble iron content was high at low phosphorus solubility and low at high phosphorus contents in the Grundy silty clay loam, the Lamoure silty clay loam with 0.05 N HCl and the Carrington loam, the Carrington silt loam, the Lamoure silty clay loam, the Muscatine loam, and the O'Neill loam with the 0.005 N HCl.

The ratio of phosphorus soluble in 0.005 N HCl to that soluble in 0.05 N HCl was calculated and is shown in table 7. The nine soils may be divided into two groups on the basis of this ratio. The Grundy silty clay loam, the Lamoure silty clay loam, the Marshall silt loam, the Grundy

silt loam and the Muscatine silt loam belong in a group with an average ratio of 0.9. The Carrington silt loam, the Tama silt loam, the O'Neill loam and the Carrington loam are in a group with an average ratio of 0.4. In three of the five soils, where the ratio was 0.8 or above and of about the same magnitude in the limed and unlimed soils there was only little crop response to rock phosphate. On the other hand, there was usually a response to phosphate fertilizer where the ratio was lower than 0.4 or where there was a wide difference in the value of the ratio in the untreated and limed soil. The soils of the first group contain a higher percentage of colloidal material and a lower amount of iron soluble in 0.05 and 0.005 N HCl than those in the second group. Apparently the phosphorus was present in the absorbed form in the soils with a ratio of 0.9 and mainly as iron phosphate, especially at lower pH values, in the group with a ratio of 0.4.

The addition of lime to the soils in the tumbler experiment brought the reaction of all soils to approximately the same pH as shown in table 8. The pH varied from 7.92 to 8.26. The addition of aluminum sulfate reduced the pH of all the soils considerably.

The addition of lime increased the solubility of phosphorus, especially in the acid soils and the aluminum sulfate brought about a decrease in the

TABLE 7. *Phosphorus, calcium and iron soluble in 0.005 N HCl*

Soil No.	Treatment	P in p.p.m.	Ca in p.p.m.	Fe in p.p.m.	Ratio of 0.005 N HCl / 0.05 N HCl soluble P
1	O	9.52	2459	132.5	0.44
2	L	10.35	3689	48.7	0.42
3	LRP	260.00	5738	56.3	1.09
4	O	3.72	2459	64.3	0.28
5	L	5.28	2869	23.7	0.38
6	LRP	146.00	3279	26.5	0.70
7	O	10.50	2459	24.3	0.71
9	L	14.90	3279	34.6	1.01
10	LRP	203.10	2459	36.0	1.02
11	O	175.60	5328	24.0	0.95
13	L	156.60	4508	20.5	1.15
14	LRP	395.60	5328	24.0	1.44
15	O	184.20	6558	32.7	1.27
16	RP	256.30	13116	20.5	0.82
17	O	72.80	3279	90.1	0.79
19	L	52.10	4508	90.1	0.81
20	LRP	197.40	4508	160.1	1.33
21	O	8.55	2049	180.2	0.52
23	L	30.50	3279	81.9	0.65
24	LRP	125.80	3279	150.1	1.21
25	O	7.41	2869	450.5	0.17
26	L	7.97	2459	318.0	0.27
27	LRP	106.80	3279	386.1	0.69
28	O	4.78	2459	257.4	0.43
29	L	3.55	3689	84.4	0.24
30	LRP	93.41	4508	337.8	0.81

TABLE 8. *The pH and phosphorus soluble in 0.002 N H_2SO_4 in tumbler experiment*

Soil No.	Treatment	Check		Soil + 4 times lime requirement		Soil + 1% $Al_2(SO_4)_3$	
		pH	p.p.m. P	pH	p.p.m. P	pH	p.p.m. P
1	O	5.23	18.3	8.00	22.4	4.09	14.5
2	L	7.27	29.5	8.13	26.2	5.58	19.6
3	LRP	7.70	241.4	8.11	213.0	5.93	261.7
4	O	5.79	15.9	7.97	21.7	3.93	16.0
5	L	7.25	30.7	8.22	26.9	4.77	15.0
6	LRP	7.64	114.3	8.25	157.4	5.02	183.1
7	O	5.18	29.1	7.98	28.7	3.92	24.6
9	L	5.89	22.4	8.04	339.0	4.35	18.3
10	LRP	6.14	164.7	8.14	152.2	4.35	188.0
11	O	5.96	99.8	8.00	80.0	4.78	95.9
13	L	6.69	89.8	8.06	56.6	5.47	82.4
14	LRP	6.14	291.1	7.97	217.2	4.95	258.9
15	O	8.18	78.7	8.16*	81.1	6.58	80.1
16	RP	8.29	83.2	8.16*	50.1	7.26	85.2
17	O	6.01	54.1	8.00	39.3	4.35	44.5
19	L	7.62	47.8	8.03	41.5	4.57	27.2
20	LRP	7.87	120.9	8.05	137.1	4.82	151.1
21	O	5.12	20.8	7.92	33.8	4.19	22.5
23	L	6.53	67.5	8.02	89.8	4.61	30.0
24	LRP	5.72	107.2	7.99	153.0	4.39	133.4
25	O	5.92	44.2	8.26	28.6	4.34	14.7
26	L	7.90	44.8	7.99	33.7	5.15	20.0
27	LRP	7.92	113.4	8.21	169.9	4.93	160.1
28	O	6.05	20.3	8.10	27.3	4.40	13.7
29	L	7.88	30.1	8.05	28.4	4.43	23.7
30	LRP	7.86	134.4	8.12	172.0	5.80	164.9

* No lime added.

phosphorus soluble in 0.002 N sulfuric acid, except in the soils which had been treated with rock phosphate in the field experiments.

DISCUSSION OF RESULTS

Certain representative biological and chemical methods proposed for the determination of the available phosphorus in soils were employed in these experiments and serve as a test of some of the assumptions upon which most methods are based. It has been quite generally assumed by the proponents of the biological methods that the phosphorus available to soil microorganisms is also available to the higher plants. The assumption may be correct but it does not necessarily follow that the growth of the microorganisms is proportional to the amount of phosphorus they may obtain. There is evidence that this may not be the case (15) (18). However, if it were the case, there seems little evidence that all the phosphorus available to *A. niger* is available to all plants. The response to fertilization was different with oats and clover than with corn. It may be pointed out further that there are also a number of factors other than phosphorus supply which govern the growth of these microorganisms and some of these factors are beyond control. The biological methods for diagnosing the

phosphorus needs of soils are of limited value and cannot be recommended for general use at present.

It is well known that the concentration of phosphorus in the soil solution is low. Teakle (17) observed that it was usually within the limits of 0.05 to 10 p.p.m. It is evident that a reserve supply of phosphorus which passes into solution readily is necessary to maintain a constant supply of the phosphate ions in the soil solution and that the rate of solution and ionization is important. Some plants require more phosphorus than others and it is conceivable that the rate of solution of phosphorus in a given soil might be adequate for some plants and inadequate for others. Therefore, the concept of availability which is often incorrectly used as synonymous with solubility should take into consideration the rate of solution of the phosphate and the plant requirements.

The rate of solution of soil phophorus is dependent upon a number of factors but chiefly upon the form in which the phosphorus occurs. The forms of phosphorus in the soil are complex but for a given soil largely determined by the reaction of that soil. Until quite recently it was commonly held that the phosphorus in the soil existed in the form of iron, aluminum, calcium, and magnesium phosphates, but the work of Mattson (6), Mattson and Pugh (7), Scarseth (14), and Ravikovitch (12) shows that much of the soil phosphorus is absorbed in the organic and inorganic colloidal complexes. The dissociation of the phosphate depends to a considerable extent upon the relative proportion of the various constituents making up these complex systems.

The observation that the amount of phosphorus dissolved by the carbonic acid solution was correlated with the pH of the soil seems to be significant. Numerous experiments have shown that liming acid soils generally results in an increased availability of phosphorus, and a rather close correlation between pH and availability of phosphorus might be expected.

Solubility under certain conditions is undoubtedly closely related to availability and there is some basis for using a 0.002 N sulfuric acid or a 1 per cent citric acid solution as the solvent. Since the phosphorus may exist in the soil in a number of forms and these forms may vary in the same soil under different conditions, it is not likely that a simple method based on solubility will ever solve the problem. The approach of von Wrangell (22) and more recently the work of McGeorge (8) in determining the rate of solubility of soil phosphorus seems most logical. Also, the work of Fisher and Thomas (3) in determining the forms of phosphorus in the soil promises much help in the solution of the problem of the selection of a method for determining available phosphorus in soils.

SUMMARY

Thirty soils from nine cooperative fields of known crop response to phosphate fertilizers were sampled and the "available" phosphorus determined by four biological and five chemical methods. No one method employed indicated accurately the phosphate needs of all the soils tested. The different crops responded differently to the application of a phosphate fertilizer. The solubility of phosphorus in carbonic acid at 25° C. and 50 mm. of water pressure appeared to be related to the pH of the acid soils. In the heavier soils where the phosphorus apparently was in the

absorbed or exchangeable form the Bray test was fairly indicative of the phosphorus needs of the soil. In general these results indicate a close relationship of phosphorus availability to the form of phosphorus in the soil and its rate of solution. It also appears that the plant needs for phosphorus must likewise be considered.

LITERATURE CITED

1. Bray, R. H.
1929. A field test for available phosphorus in soils. Ill. Agr. Exp. Sta. Bul. No. 337.

2. Dyer, B.
1894. The analytical determination of probably available mineral plant food in soils. Jour. Chem. Soc. (London), 65:115-167.

3. Fisher, R. A., and R. P. Thomas
1935. The determination of forms of inorganic phosphorus in soils. Jour. Amer. Soc. Agron., 27:863-873.

4. Hardy, F., and A. H. Lewis
1929. A rapid electrometric method for measuring lime requirement of soils. Jour. Agr. Sci., 19:17-25.

5. Hibbard, P. L.
1933. Estimation of plant available phosphorus in the soil. Soil Sci., 35:17-28.

6. Mattson, S.
1930. The laws of soil colloidal behavior: III. Isoelectric precipitates. Soil Sci., 30:459-495.

7. Mattson, S., and A. J. Pugh
1934. The laws of soil colloidal behavior: XIV. The electrokinetics of hydrous oxides and their ionic exchange. Soil Sci., 38:299-313.

8. McGeorge, W. T.
1932. Electrodialysis as a measure of phosphate availability in soils and the relation of soil reaction and ionization of phosphates to phosphate assimilation. Ariz. Agr. Exp. Sta. Tech. Bul. No. 38.

9. Mehlich, A., E. B. Fred and E. Truog
1934. The Cunninghamella plaque method of measuring available phosphorus in the soil. Soil Sci., 38:445-458.

10. Neubauer, H., and W. Schneider
1923. Die Nährstoffaufnahme der Keimpflanzen und ihre Anwendung auf die Bestimmung des Nährstoffgehalts der Böden. Zeitschr. f. Pflanzenernähr. Düng. u. Bodenk. A, 2:329-363.

11. Niklas, H., H. Poschenrieder and J. Trischler
1930. Die Kultur des Schimmelpilzes *Aspergillus niger* zur biochemischen Bestimmung der Kali-u. phosphorsäuredüngebedürftigkeit der Böden. Die Ernährung der Pflanze B, 26:97-103.

12. Ravikovitch, S.
1934. Anion exchange: I. Adsorption of the phosphoric acid ions by soils. Soil Sci., 38:219-239.

13. Salter, R. M., and J. W. Ames
1928. Plant composition as a guide to the availability of soil nutrients. Jour. Amer. Soc. Agron., 20:808-836.

14. Scarseth, G. D.
1935. The mechanism of phosphate retention by natural alumino-silicate colloids. Jour. Amer. Soc. Agron., 27:596-616.

15. SMITH, F. B., P. E. BROWN AND H. C. MILLAR
1935. The assimilation of phosphorus by *Aspergillus niger* and Cunninghamella sp. Jour. Amer. Soc. Agron., 27:988-1000.

16. SPURWAY, C. H
1925. Some factors influencing the solubility of phosphorus in soil-acid phosphate mixtures. Soil Sci., 19:399-405.

17. TEAKLE, L. J. H.
1928. Phosphate in the soil solution as affected by the reaction and cation concentrations. Soil Sci., 25:143-162.

18. THOMPSON, L. G., JR., AND F. B SMITH
1931. The effect of phosphorus on nitrogen fixation by Azotobacter. Ia. Acad. Sci. Proc., 38:299-302.

19. THORNTON, S. F.
1931. Experiments with the Neubauer method for determining mineral nutrient deficiencies in soils. Jour. Amer. Soc. Agron., 23:195-208.

20. TRUFFAUT, G., AND H. BEZSSONOFF
1928. Methode bacteriologique permettant la determination du taux d'assimilabilite des phosphates. La Sci. du Sol., 7:1-38.

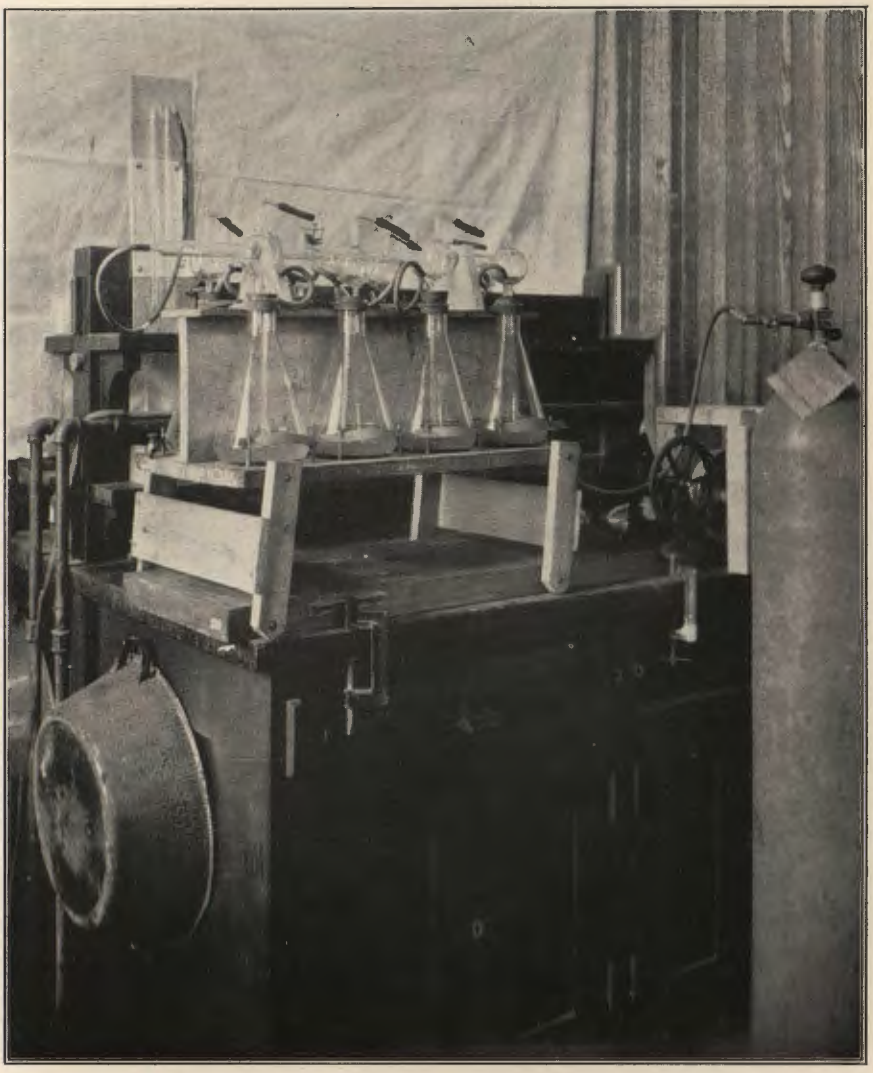
21. TRUOG, E.
1930. The determination of readily available phosphorus of soils. Jour. Amer. Soc. Agron., 22:874-882.

22. WRANGELL, M. VON
1926. Über Bodenphosphate und Phosphorsäurebedürftigkeit. Landw. Jahrb., 63:627-668.

PLATE I

Apparatus for shaking soils in water saturated with carbon dioxide.

PLATE I



MIGRATION OF SHORE BIRDS AT GOOSE LAKE, HAMILTON COUNTY, IOWA DURING THE FALL OF 1936

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With the presentation of this paper the writers hope to make an additional contribution to shore bird investigations in Iowa. The data upon which this contribution is based were collected at Goose Lake, Hamilton County, Iowa, from August 21 to November 8, 1936. Correlative observations from other localities are included in the discussion of individual birds.

Goose Lake, typical of the Wisconsin Drift marshes, is located one-half mile east of Jewell, Iowa. In respect to the mass distribution of the marshes and lakes of northern Iowa, Goose Lake, being one of the most southern, appears near the apex of the group. It is about 80 acres in area. During the period of observation not more than 20 acres of the marsh were in open water, and the remainder was grown up to a *Scirpus-Typha* (bulrush-cat-tail) associes. Three hundred yards of open shore line on the east margin of the marsh provided the waders with beaches and mudflats. This shore line was apparently maintained by the watering and feeding of cattle, domestic ducks and domestic geese. The Wilson Snipe *(Capella delicata)* preferred three acres of wet meadow adjoining the open shore line on the south and two acres of similar range around the northeast margin of the marsh.

Some preliminary shore bird observations were made on August 21 and 25. These were followed by accurate counts at two-day intervals from September 2 to November 8. The included flight graphs are based upon these counts.[1] One writer checked on the other in the field, and necessary verifications were made by reference to specimens collected in northwest Iowa and deposited in the Iowa State College Museum by L. J. Bennett and Gerald B. Spawn. No specimens were collected during the course of these investigations.

Twenty-three species of birds are discussed here in sequence according to their order in the American Ornithologist's Union check-list (1931).

SEMIPALMATED PLOVER

Charadrius semipalmatus Bonaparte

One of these birds was observed on September 22. It is considered a fairly common spring and fall migrant. Conclusive evidence of this has been presented for northwest Iowa by Bennett (1934) and Spawn (1935).

KILLDEER

Oxyechus vociferus vociferus (Linnaeus)

A common migrant and summer resident. A total of 613 birds were observed from August 21 to October 30. Counts were taken only in the

[1] Thanks are due Dr. A. E. Brandt of the Department of Mathematics at Iowa State College for advice in connection with the preparation of the graphs.

immediate vicinity. The peak of migration occurred between September 8 and 14 (fig. 1).

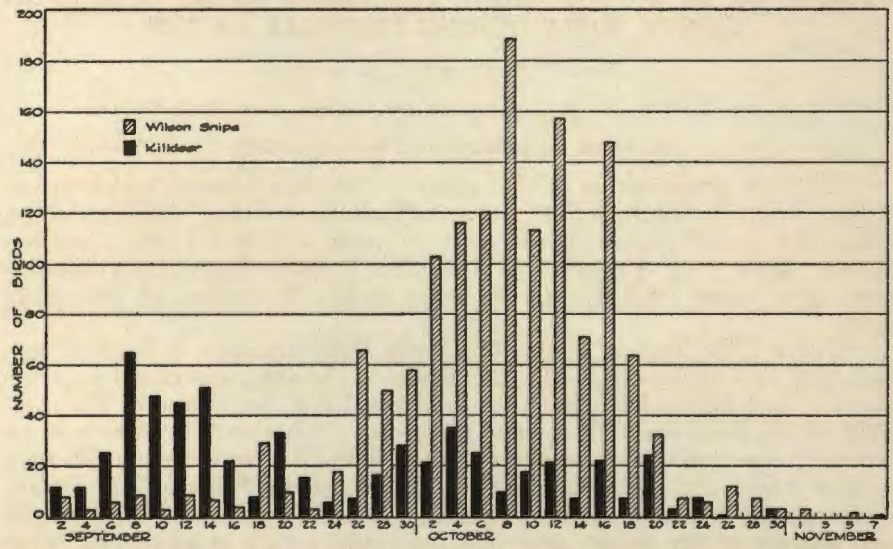

Fig. 1. Fall (1936) flight of the Wilson Snipe and Killdeer at Goose Lake, Hamilton County, Iowa.

AMERICAN GOLDEN PLOVER

Pluvialis dominica dominica (Müller)

This bird is considered an uncommon migrant. Single birds were observed on September 20 and November 8. The marsh was frozen over and several inches of snow were on the ground when the last observation was made.

Twenty of these birds spent the day in and around a closely-grazed meadow about nine miles directly north of Ames on October 24. The birds were flushed repeatedly, but they continued to return to the meadow. At dusk they took to the air of their own choosing, and after circling the area several times they disappeared from sight.

BLACK-BELLIED PLOVER

Squatarola squatarola (Linnaeus)

This bird is generally listed as a rare migrant. A single bird was recorded during the fall of 1934 on Lost Island Lake, Clay County (Spawn, 1935). Eleven birds were observed at Goose Lake from September 18 to October 26. On October 22 a flock of five was seen wading about in the shallow water along the east margin of the marsh. They appeared to be picking up food from the surface of the water. Later they were flushed from the short grass of the meadow adjoining the east shore.

RUDDY TURNSTONE

Arenaria interpres morinella (Linnaeus)

A lone bird of this rare species was observed on four consecutive dates from September 4 to 10.

AMERICAN WOODCOCK

Philohela minor (Gmelin)

On July 20, Dr. H. M. Harris observed three birds in the wooded preserve north of the Iowa State College campus at Ames. Scott observed a single bird in the same locality on July 21 and 23.

WILSON'S SNIPE

Capella delicata (Ord)

An aggregate of 1,452 of these birds was observed from August 21 to November 8. A single bird was found hiding in the sedges on November 8 after the marsh had frozen over. A count of 189 birds was made on October 8 on five acres of wet meadow (fig. 1). This moderately grazed meadow was preferred to areas supporting rank growths of bulrushes and cat-tails. The meadow vegetation was from 6 to 18 inches tall and stooled throughout. The hoof prints of stock about the meadow provided probing areas. A suitable balance of food and shelter seemed to have been provided.

Mrs. Harold Peasley and Scott observed a single bird of this species at a flowing spring along Beaver Creek near Johnson Station in Polk County on January 4, 1937.

SPOTTED SANDPIPER

Actitis macularia (Linnaeus)

Although this bird is a common migrant and summer resident throughout the state, only three birds were observed at Goose Lake. The dates for these records are: August 25, September 2 and September 6.

EASTERN SOLITARY SANDPIPER

Tringa solitaria solitaria Wilson

A fairly common migrant throughout the state. A single bird was noted on September 6. The mud flats of the Des Moines River may have proved more inviting to this bird along with others such as the Semipalmated Sandpiper, Semipalmated Plover, Spotted Sandpiper and White-rumped Sandpiper.

GREATER YELLOW-LEGS

Totanus melanoleucus (Gmelin)

Although not so numerous as the Lesser Yellow-legs, this bird is listed as a fairly common spring and fall migrant (DuMont, 1934). Fifteen of

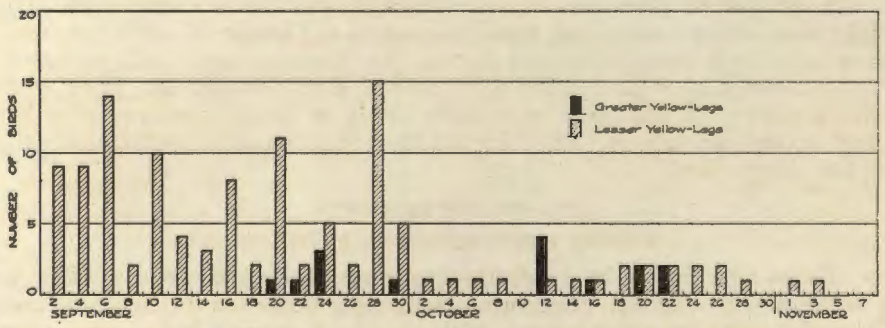

Fig. 2. Fall (1936) flight of the Greater Yellow-legs and the Lesser Yellog-legs at Goose Lake, Hamilton County, Iowa.

these birds were observed from September 20 to October 22 (fig. 2). The feeding of this bird proved quite comical. On several occasions it was observed running in small circles with the bill dipped in the water and the long legs kicking out behind as if in pursuit of some aquatic insect.

LESSER YELLOW-LEGS

Totanus flavipes (Gmelin)

A total of 146 of these birds was seen during the period of observation (fig. 2). One crippled bird remained in the area from October 2 to November 3 as the only representative of the species during that time except for a single bird which accompanied it from October 18 to 26. On September 28, an exceptionally cold day, the writers encountered 15 unusual appearing shore birds. The birds proved to be Lesser Yellow-legs with their necks pulled in, bodies slunk down and feathers fluffed out in defense against the cold.

PECTORAL SANDPIPER

Pisobia melanotos (Vieillot)

A widely distributed transient. The peak of migration for this species probably occurred before observations were begun. A total of 28 birds was counted. A flight of 12 was seen on August 28.

WHITE-RUMPED SANDPIPER

Pisobia fuscicollis (Vieillot)

"A fairly common migrant in the spring and fall" (DuMont, 1934). Only one individual of this species was observed. It was observed in company with five Red-backed Sandpipers on October 24. Spawn records a single bird for Lost Island Lake during the fall migration of 1934.

BAIRD'S SANDPIPER

Pisobia bairdi (Coues)

A single bird was observed on September 22 in company with a Least Sandpiper. This fairly common migrant is not recorded as frequently in the fall as in the spring.

LEAST SANDPIPER

Pisobia minutilla (Vieillot)

A total of 40 birds was counted from August 25 to October 20. The flight was rather continuous from August 25 to October 4, with not more than four birds being observed during that time on any single occasion. No birds were seen until three were recorded on October 20. They were almost always in company with individuals or small numbers of other short-legged sandpipers. They invariably fed in shallow water along the eastern shore line.

RED-BACKED SANDPIPER

Pelidna alpina sakhalina (Vieillot)

There appeared to be a definite migration of these birds from October 8 to October 30 (fig. 3). Forty-nine birds were observed during that time. These friendly little sandpipers with the down-curved bills were quite gregarious in their feeding. The little flocks worked swiftly and efficiently

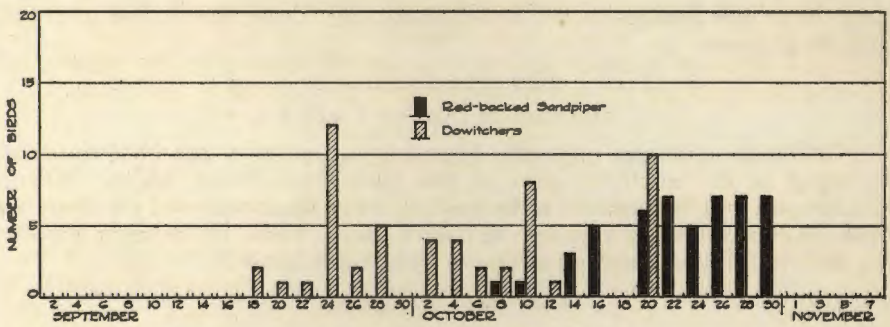

Fig. 3. Fall (1936) flight of the Red-backed Sandpiper and Dowitchers at Goose Lake, Hamilton County, Iowa.

through the shallow water of the beach, and occasionally they were seen feeding "breast deep."

EASTERN DOWITCHER

Limnodromus griseus griseus (Gmelin)

and

LONG-BILLED DOWITCHER

Limnodromus griseus scolopaceus (Say)

The impossibility of properly separating the Eastern Dowitcher and the Long-billed Dowitcher in the field makes discussion as one species necessary. Fifty-four of these birds were observed from September 18 to October 20 (fig. 3). Almost invariably the compact groups of these birds were seen feeding in shallow water along the sandy-loam beach.

STILT SANDPIPER

Micropalama himantopus (Bonaparte)

Thirty-four of these sandpipers were observed during a continuous flight from September 18 to 28. They are considered as fairly rare migrants by DuMont (1934). Eight of these sandpipers, observed on September 28, all puffed up in defense against the cold, presented an odd appearance.

SEMIPALMATED SANDPIPER

Ereunetes pusillus (Linnaeus)

A common spring and fall migrant. Eight birds were seen from September 18 to October 8. Three of these birds were recorded on October 6.

WESTERN SANDPIPER

Ereunetes mauri Cabanis

The status of this bird is undetermined (DuMont, 1934). It is reported as a common migrant by Bennett (1934) and Spawn (1935). An individual was observed on September 30.

SANDERLING

Crocethia alba (Pallas)

This rare migrant was represented by a single bird seen on September 18. It was feeding in the shallow water of the sandy-loam beach in com-

pany with two Semipalmated Sandpipers, one Least Sandpiper and two Stilt Sandpipers.

WILSON'S PHALAROPE

Steganopus tricolor Vieillot

"A fairly common migrant. Formerly a common summer resident, breeding in the northern part of the state" (DuMont, 1934). Wilson Phalaropes, which appeared to be nesting, have been reported for Dewey's Pasture and Barringer's Slough in Clay County, Iowa, by Bennett (1936). An individual was observed at Goose Lake on August 25.

NORTHERN PHALAROPE

Lobipes lobatus (Linnaeus)

"An uncommon migrant along the Missouri River Valley, rare in other parts of the state" (DuMont, 1934). On October 16, a single bird was observed from a boat about 30 yards from the east shore line. The bird was perched upon a lily pad and did not fly away until approached quite closely.

SUMMARY

1. The heavy migration of the Wilson Snipe was over at Goose Lake by October 22, 1936.
2. The Wilson Snipe exhibited a preference for wet meadows which had been moderately grazed.
3. The ratio of Greater Yellow-legs to Lesser Yellow-legs was approximately 1:10 at Goose Lake during the fall of 1936.
4. The Spotted Sandpiper, Eastern Solitary Sandpiper, Semipalmated Plover, White-rumped Sandpiper and Semipalmated Sandpiper, all common migrants throughout the state, were rare at Goose Lake during the fall of 1936.
5. Twenty-two Golden Plovers and eleven Black-bellied Plovers were seen in central Iowa during the fall of 1936.

LITERATURE CITED

BENNETT, L. J.
1934. The 1934 spring migration of shore birds through Clay and Palo Alto Counties, Iowa. Iowa State College Jour. Sci., 9:609-616.

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1936. Wilson Phalarope nesting in Iowa. Iowa Bird Life, 6:42.

DUMONT, P. A.
1934. A revised list of the birds of Iowa. University of Iowa Studies in Natural History, 15.

SPAWN, G. B.
1935. The 1934 fall migration of shore birds through Clay and Palo Alto Counties, Iowa. Iowa State College Jour. Sci., 9:617-624.

TWO METHODS FOR MEASURING EGESTION TIME FROM LARGE INSECTS

B. THOMAS SNIPES, OSCAR E. TAUBER AND HAROLD GUNDERSON

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Various standard equipments are available for collecting fecal pellets from smaller experimental vertebrate animals such as guinea pigs, rats, and mice, but all demand apparatus which is too cumbersome for, or not adaptable to, use with even large insects. To collect data on egestion time from insects such as the American roach, several of the larger grasshoppers, and older instars of silkworm and tobacco worms, two sets of apparatus have been devised of materials found, for the most part, in almost all zoological and physiological laboratories. Both methods consist, as do others built for small mammals, in the use of cages to keep test animals, and a calibrated moving surface for collecting dropped fecal pellets.

Individual cages (fig. 1) used for insects consist of a tube of copper screen-wire, 5 inches long, 1¼ inches in diameter, and held together by several globules of spot solder. About ⅛ inch of the ends of the tube is folded over to form a "hem" so that corks may easily slip in and out of the tube. The upper end of the cylindrical container is closed with a cork through which extends a "J"-shaped watering tube, the lower end of which is flared to form a cup about 7/16 inch in diameter at its widest part. The lower end of the screen tube is left open and fits into a rack (fig. 2, F) whose lower surface is covered with ¼-inch mesh galvanized iron screen. Cages are held vertically in the rack by a second board through which the upper ends of the containers protrude. The two boards with the cages are held in position by clamps on ringstands (fig. 2, C). Below the cages is the platform for collecting egested pellets (fig. 2, H).

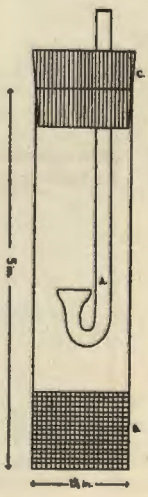

Fig. 1. Diagram of special cage used for egestion time studies. A = "J"-shaped watering tube; B = copper screen-wire; C = cork at upper end of cage through which watering tube extends.

Two types of moving platforms have been used. One employs revolving circular disks of card-board or pressed wall-board fastened to the vertical axle of an ordinary clockworks kymograph (fig. 2, J). The kymograph drum is left on the axle to give additional support to the disk. Speed of the clockworks can easily be regulated so that one revolution of the disk occurs in about 16 hours. The surface of the moving disk is marked off in hours and minutes by radii of the circular area. The calibrated surface may be covered with cellophane which can be kept clean by wiping with a damp cloth. Disks of various diameters are used, depending on the laboratory space available. Smaller ones —about 2 feet in diameter—are made of card-board; the largest—4 feet in

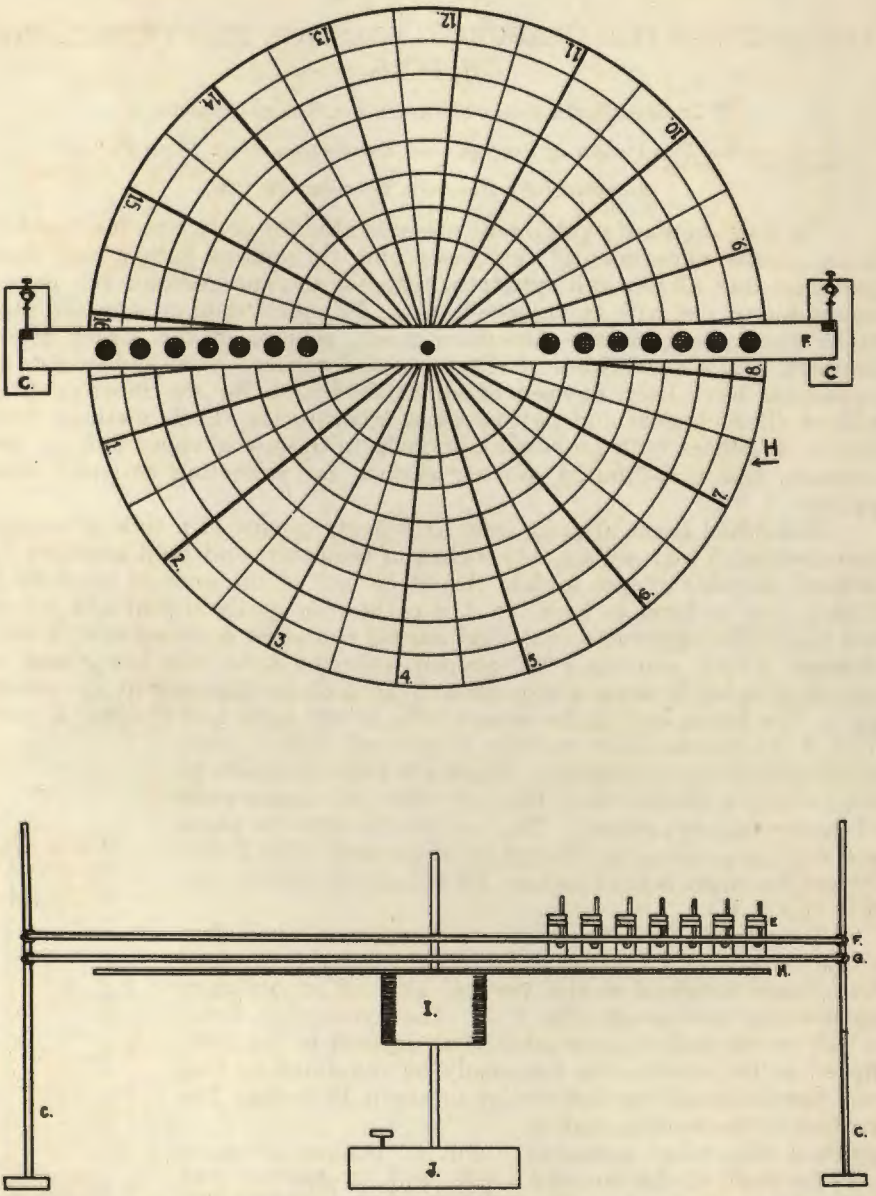

Fig. 2. Top and side views of disk type of apparatus for collecting fecal pellets. C = ringstand; E = cages; F and G = rack for holding cages, G with holes covered with ¼-inch mesh; H = pressed wall-board disk with calibration radii; I = kymograph drum to support disk; J = kymograph clockworks.

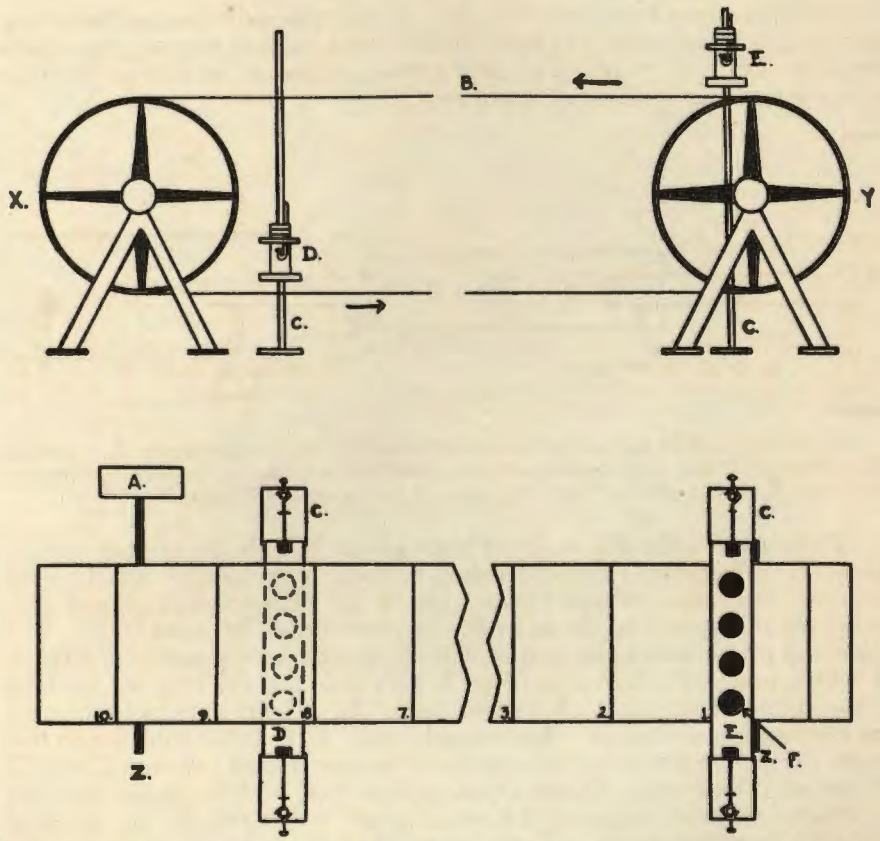

Fig. 3. Side and top views of belt type of apparatus for collecting fecal pellets. A = "Telechron" motor; B = continuous belt of kymograph paper; C = ringstands; D = lower row of cages; E = upper row of cages; F = ¼-inch mesh covering holes in lower part of rack; X = motor driven cylinder; Y = freely moving cylinder; Z = axles of cylinder.

diameter—is made of wall-board covered with white paper on which calibration radii were marked.

The second type of collecting device consists of a continuous belt of glazed kymograph paper, 8 inches wide, supported by two kymograph cylinders (X and Y, fig. 3) on horizontal axles. One of the drums is disengaged from the kymograph driving wheel and rides freely in its original bearings; the other is powered with a "Telechron" electric-clock motor (fig. 3, A) regulated so that the revolving drums make one revolution in 2 hours. The paper belt is calibrated with cross lines for hour, ½ hour, and ¼ hour intervals. The moving belt of paper can be of various lengths. The longest attempted so far is 20 feet, which allows about 9½ feet of recording surface before the belt turns to make the lower part of its

circuit. When long belts are used, they must be supported at points along their length by cross bars of some type to take care of the sagging which cannot be avoided. Sections of glass tubing supported by clamps on ring-stands serve very well for this purpose.

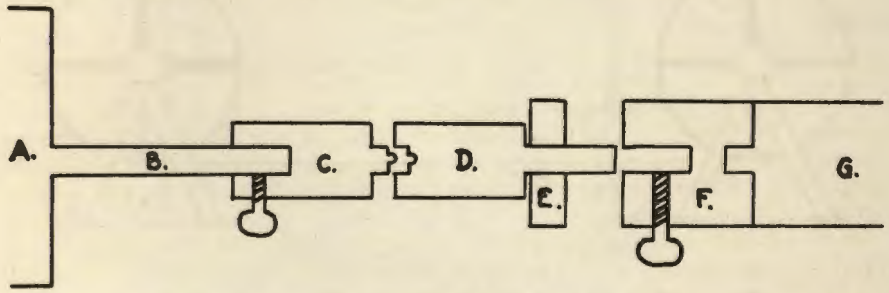

Fig. 4. Drive-shaft for moving axle of cylinder X in fig. 3. A = motor; B = driving axle of motor; C and D = sections of drive shaft which can be separated whenever necessary; E = bearing; F = "cap" fitting on D; G = axle of cylinder X.

To turn the axle (fig. 2, Z) of kymograph X with the electric motor, A, several attachments were necessary to make a drive-shaft which could be easily separated. A metal "cap" (fig. 4, C) with cross-ridge and centering pin is fastened to the axle, B, of the motor, A, by a set-screw. The ridge and pin fit into a slot and central depression at the end of a section, D, which passes through a bearing, E, screwed into the original opening of the kymograph stand. A second "cap," F, is held with set-screw on the kymograph end of D. This second "cap," F, is fitted into the kymograph axle, G, in the same ridge and slot manner found between C and D. When not in operation, C and D are pulled apart and the paper belt can be rotated without stripping the set of gears which reduce the speed of the shaft from the motor. (These speed-reducing gears are a part of the motor as purchased from the manufacturer.)

In preparation for the tests, the insects are starved for a short time (the length of time depending on the species), then placed in individual vials and, for one hour, are allowed access to food which has been mixed or covered with a colored material such as carmine, congo red, or ultramarine blue. Time of first ingestion is noted. After feeding, specimens are placed in individual cages above the pellet collecting mechanism which is then set in motion. Records are kept of times when egestion occurs. Dropped pellets are broken up in a mortar and examined microscopically for presence of dye. The time elapsing between first ingestion of dye and first appearance of the colored substance in the feces is designated as "egestion time."

DISCUSSION

Methods described above for measuring egestion time have been especially successful with the American cockroach, *Periplaneta americana*. Dimensions of the cage described have been selected particularly for this insect. The screen containers are long enough so that the internal space is not too confining even for adult specimens. It is high enough so that the insect's antennae need not be twisted or bent; it is large enough so

that a contained individual can easily move about on the screen-wire which gives a suitable foothold. The diameter, however, is such that the animal does not easily fit across the bottom or top of the cage. This situation forces the specimen to take a position usually parallel to the long axis of the cage. Since many insects prefer to keep the anterior part of the body upward when on a vertical surface, this is an advantage in the arrangement because fecal pellets can then drop downward through the bottom mesh. Occurrences of ecdysis of nymphs and oviposition of adult females seem evidence that the confinement is not particularly disturbing. For other insects, obvious modifications in cages are necessary. Smaller insects will demand smaller unit dimensions of the mesh at the bottom of the tube of screen-wire. For many leaf eating larvae, the water tube may be omitted.

Distance between cages on the supporting racks can be varied, but about ¾ inch, which allows enough space between containers for handling and working, has been found satisfactory.

Carrying capacity of the apparatus may be increased at least two ways. First, if food colored with only one dye is fed, the data on the collecting surfaces may be checked at intervals of about 8 hours so that no part of the collecting surface goes under more than one set of cages before observations are made. Second, if one set of animals is fed food with one dye, and a second set fed food with a different dye, double racks of containers may be used. Microscopic examination of the broken pellets will show which individual is responsible for a pellet located at a certain point on the collecting device, should rack arrangements be such that two animals have passed over the same section of the calibrations.

SUMMARY

1. Two methods are described for making measurements of egestion time from large insects. Insects are confined in special cages above a moving calibrated surface which consists either of a revolving disk turned by kymograph clockworks, or a continuous belt of paper supported by two horizontal cylinders, one of which is powered by a geared-down electric motor.

2. Preparatory methods of feeding test animals are given.

3. Suggestions are listed for varying the cages for different species of insects, and for increasing the carrying capacity of the mechanisms.

4. Four figures showing details of construction are included.

TEMPERATURE PREFERENCE OF THE FIREBRAT, THERMOBIA DOMESTICA (PACKARD). (THYSANURA)

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The firebrat, *Thermobia domestica* (Packard), as its common and scientific names imply, prefers remarkably high temperatures. Oudemans (1889) stated that in Amsterdam these insects were termed "Ovenvogeltjes" (little oven-birds) because of their occurrence about the ovens of bakeries. Oudemans found that his captured specimens died at room temperatures even in summer; but he was able to induce others to live and molt in an incubator at 30° Centigrade. Spencer (1930) stated that firebrats require temperatures between 90° and 110° Fahrenheit (equivalent to about 32° to 43° C.) in order to thrive. The writer (Adams 1933) began to culture firebrats at high constant temperatures, around 37° C. The cultures begun in 1931 continue to thrive and increase at the time of writing in 1937.

In order to determine the temperature preferences of these insects more accurately the writer constructed a thermal-gradient apparatus to test their thermotropism. The preliminary results confirmed Spencer's statement as to the range of the preferred temperatures and indicated that within this range the animals much prefer temperatures between 36° and 39° C. A description of the apparatus was placed in the author's doctoral thesis (1935) of which the abstract is to be published (Adams 1937). The investigation was resumed in 1936, the apparatus was improved and new data were obtained. The results of these more recent studies are presented here together with a description of the apparatus, which, with modifications, might be adapted to the investigation of the thermotropism[1] of small animals of many kinds.

A THERMOTROPOMETER DESIGNED FOR THE FIREBRAT

The final form of the apparatus is illustrated in Fig. 1. The principal part is a tin-plated sheet-metal trough 48 inches in length, 5 inches in height and 8 inches in width. Vertical sliding partitions made of the same material divide the interior into twelve chambers. The partitions project slightly above the rim of the trough. Each chamber has a glass lid and a fibre-board cover. A thin asbestos mat fitted into the bottom of the trough serves as floor covering for the chambers. Slightly above the floor of each chamber is a small circular opening through which a thermometer is inserted so that the bulb rests near the center of the floor of the chamber. Supported well above the thermometer in each chamber is a shallow dish, about three and one-half inches in diameter, for the humidity-controlling salt solution. A glass vial about one-quarter inch in diameter, filled with water and plugged with a small cotton wick, lies in each chamber to provide additional moisture for ovipositing females. A wad of ab-

[1] The term thermotropism is used here to designate movement in response to an external heat stimulus.

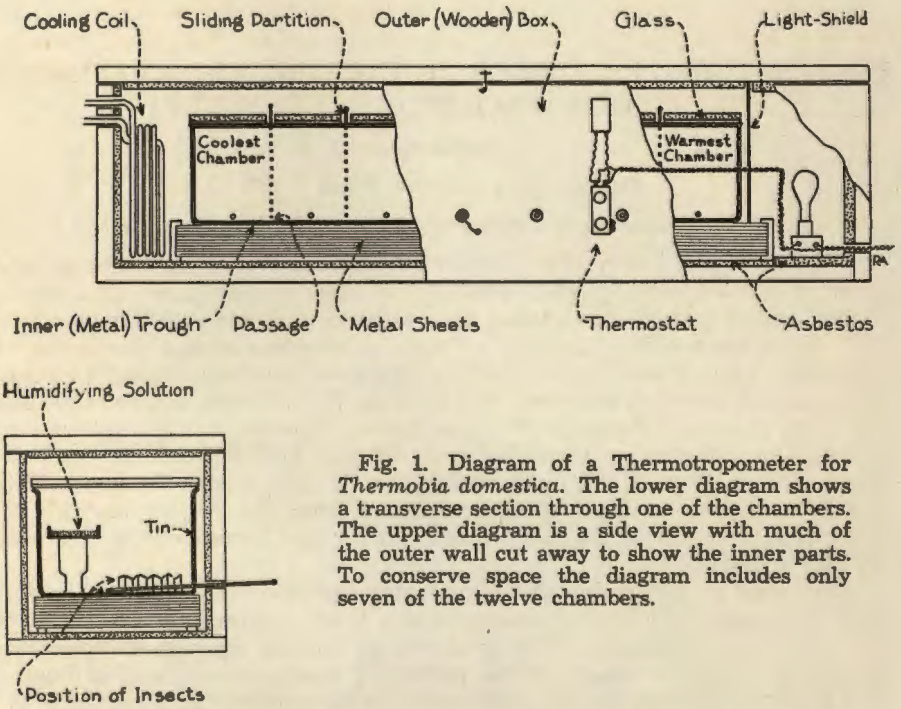

Fig. 1. Diagram of a Thermotropometer for *Thermobia domestica*. The lower diagram shows a transverse section through one of the chambers. The upper diagram is a side view with much of the outer wall cut away to show the inner parts. To conserve space the diagram includes only seven of the twelve chambers.

sorbent cotton about half-an-inch in diameter is added for the reception of eggs. Without these latter provisions mature females are likely to be restless and exhibit wanderings not traceable to the temperature factor. Food for the insects, oat-flour and dried milk, is sprinkled on the floor of the chamber and two strips of paper about one inch in width and six inches in length are plaited transversely and stood on edge as resting places. The sliding partitions between the chambers come to rest upon nail-like bits of wire each of which is manipulated from the outside of the apparatus by a slender copper wire which passes through the same opening as does the thermometer. These removable supports are of such thickness that when they are in place there is a slit-like opening between the lower edge of the partition and the asbestos mat just large enough to allow the firebrats to pass under the partition freely. When the supports are withdrawn the partitions drop down of their own weight.

The trough rests upon twenty sheets of galvanized iron two inches wider and several inches longer than the trough. The metal sheets are clamped together as a heat-distributing unit. The whole is encased in a wooden box with one-inch walls. The box is lined with asbestos and fibre-board insulation. An electric light bulb for heating the chambers is mounted within the wooden box at one end. A DeKhotinsky thermostat is mounted on the outside of the box with its bimetallic spiral projecting through the walls of the box and the trough into the second chamber. The thermostat is set in the circuit to the lamp. The light rays of the lamp are excluded from the trough by a metal shield. Within the box at the oppo-

site end of the trough is a coiled copper tubing through which water may be circulated to cool that end of the apparatus. This feature was seldom needed in practice. The hinged lid of the box is fitted to exclude light.

The apparatus is designed to provide living conditions for the firebrats over periods of weeks. To prevent distortion of the results by overcrowding of the central chambers the number of insects used is limited to about fifty adults. The partitions are set upon their metal supports so that the insects may be free to move throughout the length of the gradient of temperatures. Firebrats are characteristically unable to climb upon the smooth metal walls but must remain upon the asbestos mat and the plaited paper strips close to the bulbs of the thermometers. At intervals of four or more hours the distribution of the insects in response to temperature is obtained in this way: The supports of the sliding partitions are quickly withdrawn and the insects are thus locked in the chambers. The thermometers are read and the apparatus is then opened and the number of individuals in each chamber counted at leisure.

When, in a series of chambers, a gradient of temperatures is set up there will be a tendency for an opposite gradient of relative humidities to develop. This tendency is here largely offset by the use of saturated salt solutions containing an excess of the salt. For this purpose potassium chloride solutions are placed in each chamber. Since the apparatus is not air-tight and the air in the chambers is nearly static the relative humidities theoretically obtainable with this salt at these temperatures are not obtained. The purpose of the salt solution is, however, served when the relative humidities in the various chambers at various temperatures are brought within a few per cent of equality. Each time the apparatus is examined to record the positions of the insects the dew-point of the air in one of the chambers is measured. This instrument necessitates for the chamber a special cover partly of wood, partly of glass, with a small opening for the insertion of the instruments. As Sweetman (1933) has pointed out, it takes considerable practice to get consistent results with this method. The values obtained in the various chambers at various readings ranged between 67 and 73 per cent R. H. It may be that the actual percentages stated are subject to a more or less constant error (they are probably too low); it is their comparative uniformity which is important. Having considerable experience in the rearing of firebrats the writer believes that throughout the various chambers the insects are almost uniformly comfortable with respect to their humidity relations. When firebrats are kept in air of lower relative humidity than about 60 per cent they become attracted to the moist cotton wicks of watering vials to which they will cling for hours. Although each chamber contained such a watering vial the phenomenon was seldom seen in these experiments. That the firebrats in the thermotropometer were under generally favorable conditions is further confirmed by the reproduction which occurred during the weeks of confinement. Many of the resulting nymphs reached the fourth instar before the experiments were discontinued and the insects removed.

The firebrats used were fully grown specimens of both sexes, reared in the laboratory. For the final series of experiments, reported below, the specimens were taken from an incubator operating at about 35° C. In order to keep the number of test animals at 50 those which escaped, or were killed by the sliding partitions, were replaced by others from the laboratory stock.

RESULTS

Over 100 trials of the apparatus were made. Sixty of these were run before the apparatus and methods approximated the above description. In most of the early tests sodium chloride was the salt used for humidity control and the humidities obtained were not measured. For these reasons the data from the earlier tests are regarded as preliminary. In the summary of the first 34 trials the frequency distribution of the insects along the temperature scale was such that the greatest number of insects was recorded at 39° C. and the arithmetic mean point of the distribution was 37.5° C.

The results of the more recent trials are very similar. Some examples of the records are shown in table 1. By manipulation of the thermostat the temperature was caused to fall in the second chamber over a period of days. In the other chambers this tendency was more or less offset by a rising room temperature in trials Nos. 85 to 90. During trial No. 91 the cooling coil was operated with the result that the temperature dropped throughout most of the chambers and the insects migrated toward the remaining warmer chambers.

Fig. 2 shows graphically the summary of 41 trials. About 50 insects were run in each trial so that the figures show the distribution on the temperature scale of 2009 response-positions taken by firebrats in the apparatus. Although the temperatures indicated by the thermometers inserted into the chambers were sometimes read to half-degrees the records are here condensed to the whole-degree scale. Each sum for a half-degree

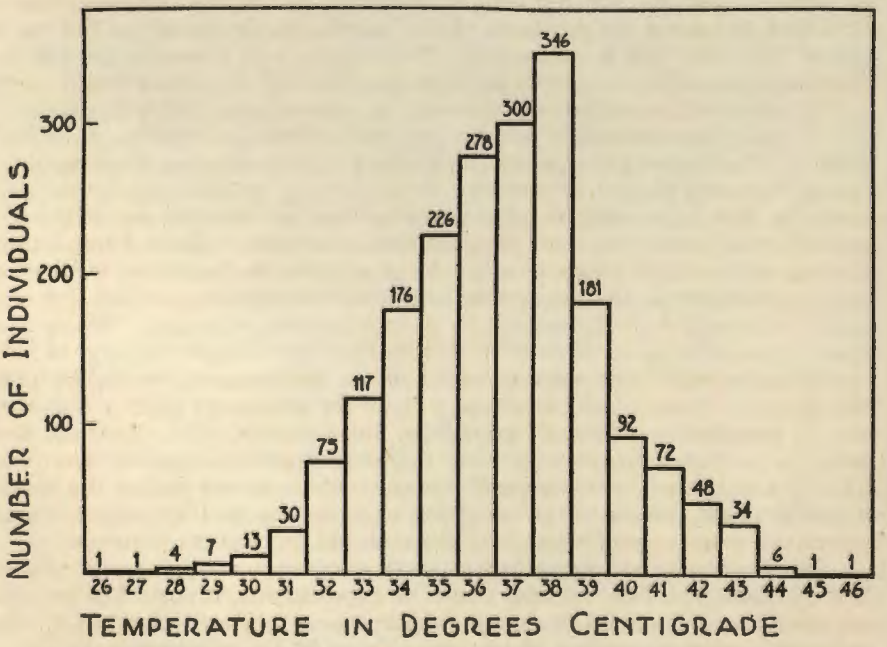

Fig. 2. Frequency distribution of 2009 positions taken by firebrats in response to temperatures in a thermotropometer. Each number written above a vertical bar is the sum of the number of insects recorded at one temperature in the apparatus.

TABLE 1. *Temperatures, humidities and self-distributions of Firebrats in eight consecutive trials in a thermotropometer*

Trial No.	Hours Since Last Reading		Individual Chambers of the Apparatus									
			XI	X	IX	VIII	VII	VI	V	IV	III	II
85	7	Tempr.	29.5	30	31	32	33.5	35	37.5	41.5	46.5	54
		Rel. Hum.	.....	.....	.....	.....	.....	70%	.....	.....	.....	.....
		No. Insects	0	0	0	3	2	10	33	3	0	0
86	17	Tempr.	29	30	30.5	31.5	33	35	37.5	41.5	46.5	54
		Rel. Hum.	.....	.....	73%	.....	.....	.....	.....	.....	.....	.....
		No. Insects	0	0	0	3	2	18	24	4	0	0
87	5	Tempr.	30	30.5	31	32.5	34	36	38	42	47	55
		Rel. Hum.	.....	.....	.....	.....	.....	.....	.....	.....	73%	.....
		No. Insects	0	0	0	3	2	8	35	3	0	0
88	5	Tempr.	31	32	32.5	33.5	35	36	38.5	42	46	53
		Rel. Hum.	.....	.....	.....	.....	.....	.....	70%	.....	.....	.....
		No. Insects	1	2	2	1	4	11	27	2	1	0
89	11	Tempr.	31.5	32	33	34	35	36.5	38.5	42	46.5	53
		Rel. Hum.	.....	.....	.....	.....	72%	.....	.....	.....	.....	.....
		No. Insects	0	2	1	2	1	18	25	2	0	0
90	6	Tempr.	32	32.5	33	34	35	36.5	39	42	46	51.5
		Rel. Hum.	.....	72%	.....	.....	.....	.....	.....	.....	.....	.....
		No. Insects	1	2	0	3	3	16	23	3	0	0
91	17	Tempr.	26	28	29.5	31	32.5	34.5	37	40.5	44.5	51.5
		Rel. Hum.	.....	.....	.....	.....	.....	.....	.....	.....	73%	.....
		No. Insects	0	0	0	1	2	7	33	7	0	0
92	6	Tempr.	26	28	29	31	32	34	36.5	40	44	51.5
		Rel. Hum.	.....	.....	.....	.....	.....	.....	.....	70%	.....	.....
		No. Insects	0	0	0	0	3	5	22	20	0	0

point was split into equal (or, in the case of odd-numbered sums, nearly equal) portions, one of which was added to the sum at the whole-degree point above and one to that below. Although the modal point of the distribution is at 38° C. the arithmetic mean of the distribution is at 36.6° C.

DISCUSSION

The difference between the results in the preliminary and in the final trials is roughly a matter of one degree in temperature. The arithmetic mean of the former is 37.5° C. and of the latter 36.6° C. Much of this difference is probably due to a difference in the positions of the thermometer bulbs in the chambers in the two sets of trials. In the latter the thermometers were inserted into the chambers slightly on the cooler side of the middle of the chamber space. The error so produced is likely to have been as much as a half-degree or more in the warmer chambers. If, in view of these facts, we correct the data by an upward shift of one-half degree the arithmetic mean of the distribution moves to a point slightly above 37° C. and the point of greatest concentration becomes 38.5° C.

One of the purposes of the study was to determine the temperature most preferred by the animals. It is evident that this point should be near

the center of the thermotropic distribution. It may be debated, however, which of the types of averages is to be chosen as the best indicator of this most preferred point (at which, theoretically, the animal in the thermotropometer experiences a minimum of thermal stimuli). The definition in the earlier paper (Adams 1937) points to the mode of the distribution as the best indicator, but, since the data were limited in quantity to the results of 34 trials with 23 to 44 insects in the apparatus, the arithmetic mean, 37.5° C., which was calculated from all the data was tentatively chosen as the best approximation of the "thermotactic optimum" then available. In view of the more recent trials, however, in which again the mode is about one and one-half degrees higher than the arithmetic mean, it seems reasonable to suspect that negative skewness is a characteristic of thermotropic distribution at biologically high temperatures; and that, provided sufficient data are utilized to derive it, the mode of the distribution should be accepted as the most preferred temperature. Since the magnitude of the smallest change in temperature required to cause a measurable change of response by the animals may be any fraction of one degree Centigrade, the precision with which the most preferred temperature may be determined remains for further investigation.

While the preferred temperature, determinable with a thermotropometer, must not be confused with the general optimum, determinable only by lengthy cultural experiments, the temperature the insect prefers is probably very close to, if not identical with, the temperature at which it thrives best. An investigator setting out to study and to rear a species of insect may save himself much labor by testing some specimens in a thermotropometer and thereby quickly gaining an approximation of the temperature at which he should begin his cultures in order to get at least fairly satisfactory results. The thermotropometer will show him not only the central tendency but also the limits of the insect's temperature preferences; and, as with the firebrat, these limits are likely to correspond roughly with the cultural limits.

The preferred and the optimum temperatures are not to be confused with the temperature at which there is maximum velocity of development. According to rearing experiments (Adams 1937) the life-cycle of the firebrat from egg to egg was completed in as few as 7 to 8 weeks at 42° C. But the mortality was higher than that at 37° C., at which point the cycle required 11 to 12 weeks.

SUGGESTIONS REGARDING THERMOTROPOMETERS

Although the apparatus described above is simple and inexpensive it has several shortcomings. In accordance with the laws of heat the drop in temperature from the warmer to the cooler end of this apparatus is not uniform. Each succeeding chamber from the warmer to the cooler end covers a smaller portion of the temperature scale than the preceding one.

Since only one temperature is recorded from a chamber at a reading any one point of temperature which occurs in the warmer chambers is likely to appear in the records less frequently than a point of temperature which occurs in the cooler chambers. This probably accounts for the irregularities of the data for points above 36° C. A larger number of experiments might have given a smoother curve. Furthermore, the temperatures in the cooler chambers remote from the thermostat were in-

fluenced by the fluctuating temperatures of the room. Another criticism is that the chambers have no air-circulating system to insure uniformity of air conditions within their enclosed spaces.

In view of these criticisms the writer recommends that each chamber in a thermotropometer be equipped with its own units for the control of temperature, humidity and air-movement. Such an apparatus would consist essentially of a series of individually air-conditioned cabinets set side by side with narrow closable openings cut through the contacting sides to allow the animals to move freely from cabinet to cabinet. It would have the disadvantages of being rather complicated and costly to construct.

LITERATURE CITED

ADAMS, J. A.
1933. Biological notes upon the firebrat, *Thermobia domestica* (Packard). Jour. New York Entomol. Soc., 41:557-562.

1937. The firebrat, *Thermobia domestica* (Packard), and its gregarine parasites. Abstract of doctoral thesis. Iowa State College Jour. Sci. (in press).

OUDEMANS, J. T.
1889. *Thermophila furnorum* Rov. Tjidschr. v. Entomol., 32:425-432. 1 pl.

SPENCER, G. J.
1930. The fire brat, *Thermobia domestica* Packard (Lepismidae) in Canada. Canad. Entomol., 62:1-2.

SWEETMAN, H. L.
1933. Studies of chemical control of relative humidity in closed spaces. Ecology, 14:40-45.

STUDIES ON BROOD A JUNE BEETLES IN IOWA[1]

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White grubs are the immature stage of a moderately large genus (Phyllophaga (Harris)) of beetles known as "June beetles" or "May beetles." At the present time approximately 123 species are recorded as occurring in the United States and Canada. Various investigators in different localities report that from 10 to 30 or more species may be collected in almost any region with the exception of that area immediately along the west coast. In Iowa and other midwestern states, the larvae are considered one of the more serious pests of field and garden crops. Very often, too, the adults are destructive as they are foliage feeders and defoliate trees and other plants in certain localities. The present paper is the result of a survey of Brood A adults and brings together all the known records of this brood for the state, as well as certain field studies on stomach poisons which were carried out to parallel a series of laboratory experiments on similar materials and published in an earlier paper by Andre and Pratt (1936).

BROODS

Numerous papers are recorded in entomological literature dealing with the life history and habits of the various members of this genus. It has been determined that the length of life cycle of the different species varies from one to four years, depending on the particular species involved, climatic conditions, and the locality where they occur. In Iowa, however, it may be said that usually three years are required to complete the development from the time the egg is laid until the adult emerges to feed. As a brood appears each year they are called Broods A, B and C. During a Brood A flight year (1932 and 1935) the adults are extremely abundant. As 1935 was the year for Brood A adults to be present, it was decided that a survey should be made to determine the number of species present, the distribution of the various species, and the food plants in the different localities. It is planned that this same type of survey will be continued for Broods B and C.

METHODS

Most species of June beetles fly and feed during the nights of the spring and summer. At this time one is able to pick them by hand or shake them from the branches of their host plant onto a large canvas. A flash light was used to hand pick them from the foliage in many cases. When collections were made during the daylight it was necessary to turn back dead leaves on the ground in woodlots and timber areas thus exposing the hiding beetles.

[1] Journal Paper J446 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 128.

Each collection from its host was tied in a cheesecloth bag, labelled as to locality, date of collection, and host. The bags containing the beetles were dropped into 70 per cent alcohol, where they remained until they were mounted for study.

COLLECTIONS AND SPECIES

During the summer of 1935, collections of June beetles were made in 69 of the 99 Iowa counties. Table 1 gives the number of individuals collected from the various plants. Since collections were made in many cases from plants that were most readily reached the choice of food plants as shown in the table cannot be considered significant.

In the case of each species collected the date on which it was first taken is recorded and in most instances the localities and host plants are given. The following list is according to the total number of each species collected by various investigators up to the present time and is not arranged to show relationship or position.

Phyllophaga hirticula (Knoch)

Dubuque, Leon, McGregor, New Sharon, Ottumwa, Clarinda, Kellerton, Mason City, Keokuk, Muscatine, Ames, Waukon, Fayette, Decorah, Cresco, Osage, Lake Mills, Algona, Fort Dodge, Boone, Centerville, Bloomfield, Washington, Mt. Pleasant, Tipton, Clinton, Cedar Rapids and Tama.

Taken from willow, elm, cottonwood, ash, shingle oak, hackberry, walnut, hickory, and quaking aspen.

A very abundant form. May 6 to July 23.

Phyllophaga implicita (Horn)

This species was abundant in every county where collections were made.

It was collected from elm, willow, hazel brush, cottonwood, ash, shingle oak, white oak, hawthorne, hackberry, dogwood, gooseberry, walnut, hickory, prickly ash, quaking aspen, ragweed, thistle, curled dock, and lambs quarter.

The most abundant species found in Iowa during 1935. It could be collected in numbers from almost any willow, cottonwood or elm in the state. Found as early as March 15 and as late as July 23.

Phyllophaga tristis (Fabricius)

Waukon, Ames, Ottumwa, Bloomfield, Cedar Rapids, McGregor, Davenport, Farmington, Wapello, and Mt. Pleasant.

Taken from elm, cottonwood, ash, and white oak.

Collection of this species first made on May 18 and last on July 2.

Phyllophaga rugosa (Melsheimer)

Ames, New Sharon, Keokuk, Muscatine, Sioux City, Sidney, Marshalltown, Clarinda, Leon, Centerville, Bloomfield, Mt. Pleasant, Ottumwa, McGregor, Dubuque, Clinton, and Maquoketa.

Collected from white oak, cottonwood, hackberry, shingle oak, box elder, walnut, hickory, elm, willow, linden, and quaking aspen.

Rusoga was very abundant in all the localities where it was collected. Many more specimens than were obtained could have been gathered had they been needed. First found on April 28 and last on July 22.

Phyllophaga futilis (LeConte)

Ames, New Sharon, Keokuk, Muscatine, Sioux City, Sidney, Marshalltown, Chariton, Mason City, LeMars, Cedar Rapids, Vinton, and McGregor.

Host plants from which it was taken were wild cherry, plum, linden, elm, and bur oak.

This species was distributed rather widely over the state during 1935, but was not collected in large numbers in any one place. The first specimen was found May 10 and the last on July 16.

Phyllophaga fusca (Froelich)

Ames, Des Moines, Indianola, Ottumwa, Oskaloosa, Davenport, Muscatine, Columbus Junction, Keokuk, Boone, Farmington, Algona, Mason City, Webster City, LeMars, Waukon, Decorah, Centerville, Corydon, Leon, Mt. Pleasant, Bloomfield, Dubuque, Clinton, Grundy Center, Marshalltown, Tama, Vinton, Cedar Rapids, Tipton, Newton, Albia, and Chariton.

Host plants included elm, willow, cottonwood, plum, linden, hawthorne, white oak, and quaking aspen.

Fusca appears to be a rather common species found in widely separated areas over the state. Found as early as April 8 and as late as July 22.

Phyllophaga hornii (Smith)

Kellerton, New Sharon, Oskaloosa, Blakesburg, Bloomfield, and Leon.

Hornii was collected from hickory in all the localities where it was found during 1935, with the exception of six specimens taken at Blakesburg on shingle oak.

This species was not found in numbers at any time. It was first recorded during 1935 on May 7 and the latest date of collection was July 10.

Phyllophaga inversa (Horn)

Ames, Des Moines, Kellerton, Columbus Junction, Centerville, Ottumwa, and New Sharon.

It was collected from three host plants, namely—hickory, willow, and walnut.

Only a few individuals could be obtained from any one host plant. First noted on May 16 and last on July 13.

Phyllophaga anxia (LeConte)

McGregor, Postville, Decorah, Bloomfield, Dubuque, Fayette, Mt. Pleasant, and Clinton.

Collections were made from willow, linden, and quaking aspen.

This species was never taken in numbers. It was first collected on June 3 and the last collection was made on July 23.

TABLE 1. *Collection data for Brood A, 1935*

Species of Phyllophaga	Sex	Elm	Willow	Cottonwood	Ash	Shingle oak	White oak	Hawthorne	Hackberry	Hazel	Dogwood
implicita (Horn)	♂	5140	3569	1268	10		6	22	11	2	1
	♀	6173	4025	1292	4		3	19	11	3	
hirticula (Knoch)	♂	3728	1156	39	12	1030			1		
	♀	3800	1151	51	13	1201					
rugosa (Mels.)	♂	1220	1128	560		112					
	♀	1349	1080	611		111	1		2		
fusca (Froel.)	♂	109	26	57			1			3	
	♀	168	13	22				1		1	
tristis (Fab.)	♂			7	1		203				
	♀	2		19	5		170				
crassissima (Blanch.)	♂		19		7						
	♀		24		16						
ilicis (Knoch)	♂				29						
	♀				12						
anxia (Lec.)	♂		11								
	♀		19								
crenulata (Froel.)	♂										
	♀										
inversa (Horn)	♂		19								
	♀		6								
drakei (Kirby)	♂		16							3	
	♀		11		2					2	
micans (Knoch)	♂				3	8					
	♀				2	7					
nitida (Lec.)	♂										
	♀										
hornii (Smith)	♂					3					
	♀					3					
vehemens (Horn)	♂				2						
	♀				4						
futilis (Lec.)	♂	6									
	♀										
balia (Say)	♂		4								
	♀		7								
bipartita (Horn)	♂						2				
	♀						3				
corrosa (Lec.)	♂							3			
	♀							5			
fraterna (Harris)	♂									4	
	♀									1	
prunina (Lec.)	♂									1	
	♀						3				
forsteri (Burm.)	♂										
	♀						2				
marginalis (Lec.)	♂									2	
	♀										
fervida (Fab.)	♂	1									
	♀										
congrua (Lec.)	♂										
	♀										
hirtiventris (Harris)	♂										
	♀										

Gooseberry	Walnut	Hickory	Prickly ash	Red oak	Quaking aspen	Linden	Plum	Birch	Cherry	Bur oak	Box elder	Other	Total ♂'s	Total ♀'s	Total	Dates occurring
	1		2	1	210							60				
6	1	1		5	169							8	10303	11720	22023	3-15 7-23
		2			58											
	2	17			60								6026	6295	12321	5-6 7-22
		3			370	19					9					
	7				298	21					27		3421	3507	6928	4-28 7-22
					10	12	4									
					4	7							222	216	438	4-8 7-22
													211	196	407	5-18 7-2
													26	40	66	6-2 7-5
						7							29	19	48	4-29 8-1
						9							20	19	39	6-3 7-23
										27						
										11			27	11	38	5-20 7-3
		3														
	2	9											22	17	39	5-16 7-13
					3								19	18	37	5-3 7-11
		4														
		9											15	18	33	6-20 7-10
						21										
						8							21	8	29	6-27 7-15
		10														
		11											13	14	27	5-7 7-10
	5															
	9												7	13	20	5-13 7-17
						2	3		1							
						1	2			3			12	6	18	5-10 7-16
											3		4	10	14	5-28 7-6
								3								
								2					5	5	10	6-10
													3	5	8	7-8 7-15
													4	1	5	6-17 7-1
													1	3	4	6-7 7-19
													0	2	2	6-27
													2	0	2	6-28
													1	0	1	6-11
	1												1	0	1	6-19
												1	1	0	1	June
										Sub Total			20416 42559	22143		

Phyllophaga vehemens (Horn)

Missouri Valley, Onawa, Sioux City, New Sharon, Farmington, Keokuk, Vinton, Cedar Rapids, and Tama.

Collected only from walnut and ash.

It was first taken on May 13 and last on July 17.

Phyllophaga drakei (Kirby)

Ames, New Sharon, Oskaloosa, McGregor, Postville, Decorah, Farmington, Fayette, Tama, Mason City, Chariton, Albia, Council Bluffs, and Farmington.

Taken from willow, quaking aspen, white oak, and hazel brush.

Collected first on May 3 and last on July 11.

Phyllophaga crassissima (Blanchard)

Keokuk, Mt. Pleasant, Columbus Junction, Muscatine, Missouri Valley, Council Bluffs, Ottumwa, and Davenport.

Collected from ash, birch, and willow.

This species was rather scarce in most localities and usually only one or two individuals could be found on any one host. Earliest occurrence was on June 2 and latest on July 5.

Phyllophaga fraterna (Harris)

Clinton, Dubuque, and Mt. Pleasant.

Occurred on but one host plant—hazel brush.

Collected on June 17 and July 1.

Phyllophaga ilicis (Knoch)

Postville, McGregor, Sioux City, Davenport, Clinton, Centerville, Keokuk, Mt. Pleasant, and Wapello.

Taken from ash and linden.

Found first on April 29 and last on August 1.

Phyllophaga micans (Knoch)

Ottumwa and Mt. Pleasant.

Micans was collected from ash and shingle oak.

Collected first on June 20 and last on July 10.

Phyllophaga crenulata (Froelich)

McGregor, Postville, Centerville, and Cedar Rapids.

Taken from the foliage of bur oak in all cases.

The earliest date of collection was May 20 and the latest July 3.

Phyllophaga prunina (Le Conte)

Ames, Ottumwa, Keokuk, and Dubuque.

This species was collected from the leaves of hazel brush and white oak.

It was found on four occasions and in each case only one specimen could be found on a plant. The earliest record of its occurrence in 1935 was on June 7 and the latest was July 19.

Phyllophaga marginalis (Le Conte)

McGregor.
Taken from hazel brush.
The only date on which this species was collected was June 28.

Phyllophaga nitida (Le Conte)

Postville, McGregor, Fayette, Ames, and Hampton.
In every case it was collected from the foliage of linden.
The earliest collection was made on June 27 and the latest on July 15.

Phyllophaga congrua (Le Conte)

Woodbury County near Sioux City.
The only specimen that could be found was on black walnut.
Apparently a scarce species as only one specimen was collected and that on June 19.

Phyllophaga balia (Say)

McGregor, Ames, Postville, and Dubuque.
It was found on two species of plants, namely—willow and box elder.
This species was collected on two occasions, May 28 and July 6.

Phyllophaga bipartita (Horn)

Farmington and Postville.
The only plant on which this species was found was white oak.
Date of collection, June 10.

Phyllophaga corrosa (Le Conte)

McGregor and Ames.
Crategus was the only plant from which it was collected.
Collected on July 8 and July 15.

Phyllophaga fervida (Fabricius)

Van Buren County, near Farmington.
The host from which it was taken was elm.
Only one specimen was found and that on June 11.

Phyllophaga forsteri (Burmeister)

McGregor was the only locality where this species was collected. It was taken from white oak foliage. Found June 27.

Phyllophaga hirtiventris (Horn)

Lakeside Laboratory, Dickinson County, Iowa.
One specimen from light trap taken by Prof. H. E. Jaques.[2]

[2] The writer wishes to express his appreciation to Prof. H. E. Jaques for furnishing this record.

Collected during June, 1935. This species has not hitherto been reported in Iowa.

DISCUSSION

As shown in table 1 a total of 42,559 beetles was collected during the spring and summer of 1935. Twenty-six species were taken although not all of them occurred in abundance. *P. implicita, P. hirticula* and *P. rugosa* were the most abundant species. During the early part of the season males were most numerous, at mid-season males and females were about equally abundant, and as the summer progressed females became the dominant of the two sexes. It is interesting to observe that out of 42,559 specimens there were 20,416 males and 22,143 females.

Elm, willow and cottonwood were in many localities almost the only available trees that could be used for sampling; for that reason a larger number of individuals was taken from these trees than from any other hosts.

More species of beetles were found in eastern and southern Iowa in any particular locality than in the western and northwestern portions of the state. No doubt, the fact that there are more species of host plants, and a larger area in permanent bluegrass pasture in the eastern and southern portions somewhat accounts for this. It is possible also that weather conditions, especially rainfall, play an important role in the distribution of the various species.

Table 2 records all the species belonging to Brood A so far as known. In column I is included the species and number of each collected by Jaques in the flight season of 1923, and published in a paper by him (1926); column II records those listed by the same writer in a paper published in 1927, and represents specimens collected by him during the flight season of Brood A in 1926. Column III records the June beetles listed by Travis (1934), and includes those specimens Jaques recorded in the two previously mentioned papers as well as many others added by Travis and other collectors. Column IV lists those taken by the writer during the flight season of 1935. Four species other than are shown in table 2 have been recorded as occurring in Iowa, but the year in which the adults were taken is not available. For that reason it is impossible to assign them to any one of the three broods. The four are *P. quercus, gracilis, ephilida,* and *spreta.*

FIELD TRIALS WITH STOMACH POISONS

Many requests are received each year by the Iowa Agricultural Experiment Station for a method of controlling imago Phyllophaga during the flight period. Trees and shrubs of various species are sometimes entirely denuded by the feeding activities of the nocturnal species. Certain field trials were made during the flight season of Brood A in 1935 in the hope of learning the relative merits of several poisons. Although there are several thousand references to June beetles in the literature of entomology only a few touch upon the control of the adults by means of insecticides. Davis (1916) mentions paris green, lead arsenate, and similar arsenicals as being effective against the beetles when sprayed on the foliage of the trees. Later, Vickery and Wilson (1919) showed that lead

TABLE 2. *Brood A records for Iowa*

Phyllophaga	1923 I	1926 II	1932 III	1935 IV	III & IV
hirticula (Knoch)	517	2,568	41,084	12,321	53,405
implicata (Horn)	31	239	9,980	22,023	32,003
tristis (Fab.)	1	9	10,647	407	11,054
rugosa (Mels.)	90	569	2,993	6,928	9,921
futilis (Lec.)	407	1,370	2,062	18	2,080
fusca (Froel.)	100	5,234	937	438	1,375
hornii (Smith)		15	560	29	589
inversa (Horn)		123	507	39	546
anxia (Lec.)		42	352	39	391
vehemens (Horn)		23	255	20	275
drakei (Kirby)	7	15	211	37	248
crassissima (Blanch.)	12	123	157	66	223
fraterna (Harris)	62	134	206	5	211
ilicis (Knoch)	8	12	140	48	188
micans (Knoch)	13	31	91	33	124
crenulata (Froel.)	6	6	53	39	92
prunina (Lec.)		2	52	4	56
marginalis (Lec.)			53	2	55
nitida (Lec.)			25	29	54
congrua (Lec.)			44	1	45
balia (Say)		3	23	14	37
bipartita (Horn)		4	14	10	24
corrosa (Lec.)			8	8	16
fervida (Fab.)		14	14	1	15
forsteri (Burm.)		1	3	2	5
villifrons (Lec.)			4		4
barda (Horn)		2	2		2
hirtiventris (Horn)				1	1

arsenate, both as a spray and dust, was rather effective against certain wingless species which were destructive to cotton plants. In Wisconsin, Fluke (1933) has demonstrated that lead arsenate sprays (2 pounds to 50 gallons water) will prevent to a large extent the defoliation of oak trees. Other than these field trials a few references deal with poison trials under laboratory conditions. Travis and Decker (1933) made a study of the value of calcium arsenate as a dust for beetles caged in a screened insectary. Concurrent with the experiments to be reported in this paper, Andre and Pratt (1936) studied the relative value of certain stomach poisons in the laboratory when compared on an M.L.D. (median lethal dose) basis. The following field tests were conducted to ascertain whether the relative values of the compounds used in the laboratory would parallel those tested under field conditions.

Four dusts—namely, paris green, acid lead arsenate, calcium arsenate, and sodium fluosilicate—were used. Each poison was mixed with bentonite so that when the dust was applied it consisted of 40 per cent poison and 60 per cent bentonite by bulk. The trees selected for the trials were bushy willows about eight feet high. Dusts were applied to the trees about five o'clock in the afternoon. A hand duster was used to apply these dusts and they were applied at two different rates: (1) A light applica-

tion, where 2 pounds of poison were applied to each tree, and (2) a heavy application, where from 5½ to 6 pounds of dust were applied to the foliage of the tree being dusted. Untreated control trees were chosen adjacent to each series of treated trees. All the trees were located on level ground in a row about a mile long and all were about the same size. The only species of beetle used in these trials was *P. implicita*. Two other species were often taken in small numbers—*hirticula* and *fusca*—but data on these are not included.

The beetles usually migrated to the willows at about 8:30 to 9:00 p. m. and soon commenced feeding. Each tree was watched to see if any particular treatment would cause the beetles to leave. It was soon evident that trees dusted with paris green, acid lead arsenate and calcium arsenate caused a portion of the beetles to leave before their normal feeding period was completed. Two hours after the incoming flight to the trees treated with paris green and lead arsenate, only approximately one-third of the beetles remained. Calcium arsenate treated willows lost about 10 per cent of their population in the same two-hour period, whereas those treated with sodium fluosilicate lost none.

To gain some insight as to the mortality obtained where the beetles left the treated trees before their normal feeding period was over the beetles were collected from the foliage as soon as they stopped feeding and prepared to fly from the trees. Although not as many could be collected in this manner as where they were hand picked after feeding for four hours, the results obtained with those collected in this manner when compared with the others offer an interesting comparison with the results from the longer feeding period.

In all other instances the beetles were allowed to feed for about four hours and were then picked from the foliage of the various trees. Each series was kept in separate boxes and marked as to the treatment they received. Then the beetles were taken to the screened insectary where they were confined in rearing cages which had a two-inch layer of moist soil in the bottom. The cages were supplied with fresh willow leaves each evening. At intervals of 24, 48, 72 and 96 hours the number of living beetles was recorded and the dead were discarded. Table 3 shows the percentage of dead beetles where the light dosages were applied and table 4 records the mortality obtained with the heavy dosages at the re-

TABLE 3. *The toxicity of four stomach poisons to June beetles, using light applications under field conditions*

Time in hours	Sodium fluosilicate		Acid lead arsenate		Paris green		Calcium arsenate		Checks	
	Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval	
	♂*	♀*	♂	♀	♂	♀	♂	♀	♂	♀
24	5.0	4.1	10.2	8.4	14.5	10.7	8.6	4.1	2.7	1.8
48	7.0	6.4	19.6	13.7	24.1	19.0	10.7	5.2	5.1	4.9
72	11.0	7.4	24.7	16.7	31.1	23.1	13.8	8.0	7.2	5.0
96	14.8	9.5	30.7	18.6	38.3	28.1	16.5	10.9	8.4	6.0

* Each column is based on a sample of 250 individuals. This applies also to table 4.

TABLE 4. *The toxicity of four stomach poisons to June beetles, using heavy applications under field conditions*

Time in hours	Sodium fluosilicate		Acid lead arsenate		Paris green		Calcium arsenate		Checks	
	Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
24	10.3	4.1	40.7	15.2	42.0	24.0	22.2	9.7	3.0	1.5
48	17.5	8.2	52.4	20.3	58.8	33.7	32.3	16.9	4.0	3.8
72	24.7	11.2	57.5	25.0	74.7	41.9	40.2	22.5	6.6	5.0
96	25.7	12.2	60.9	27.7	81.4	44.0	42.2	26.3	7.8	5.5

spective intervals of time. Table 5 shows the mortality in the case of paris green, acid lead arsenate and calcium arsenate where the beetles were picked from the foliage as they started to leave the treated trees after various lengths of periods of feeding. As is shown in this particular table irregular numbers of adults were used. In the case of table 3 and table 4, each test and check lot consists of a sample of 250 beetles.

PARIS GREEN.

Both heavy and light applications of this compound (tables 3 and 4) proved to be the most toxic stomach poison to both males and females under field conditions. With the heavy dosage 81.4 per cent of the males were dead after a 96-hour period whereas 44.0 per cent of the females died in this same interval of time. The light application resulted in a 38.3 per cent mortality in the males and a 28.1 per cent in the case of the females.

As is recorded in table 5, 103 males hand picked from the foliage just as they were preparing to leave the trees treated with the heavy application of paris green, showed a mortality of 98.0 per cent in the 96-hour period. A total of 124 females collected in the same manner died to the extent of 93.4 per cent in the same length of time. This increased mor-

TABLE 5. *The toxicity of three stomach poisons to June beetles, hand picked from foliage as they started to leave*

Time in hours	Paris green		Acid lead arsenate		Calcium arsenate		Checks	
	Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval		Percentage dead at each time interval	
	103 ♂'s	124 ♀'s	97 ♂'s	102 ♀'s	83 ♂'s	90 ♀'s	250 ♂'s	250 ♀'s
24	51.2	47.0	60.9	53.4	38.7	36.9	3.0	1.5
48	86.8	78.9	90.1	87.2	60.8	49.6	4.0	3.8
72	94.2	87.6	98.2	91.6	82.7	70.1	6.6	5.0
96	98.0	93.4	99.7	97.1	84.1	76.8	7.8	5.5

tality over the other beetles may have been partially due, at least, to the fact that the beetles received a toxic dose of poison, became sickened by it, and started to fly back to their hiding place in the soil.

It is interesting to note that paris green was also the most toxic to both males and females under laboratory conditions, and this poison was least readily eaten by them (Andre and Pratt, 1936).

ACID LEAD ARSENATE

Of the four stomach poisons compared acid lead arsenate ranked second in toxic value when employed as a dust under field conditions. With the heavy application 60.9 per cent of the males and 27.7 per cent of the females were dead after a 96-hour period.

Where the beetles were picked from the foliage (table 5) just before they left the treated trees, 97 males showed a mortality of 99.7 per cent, whereas of 102 females 97.1 per cent were dead in 96 hours.

A light application of acid lead arsenate produced a 30.7 per cent mortality in the females, and an 18.6 per cent kill in the males in a period of 96 hours. Under laboratory conditions Andre and Pratt (1936) found acid lead arsenate more toxic than sodium fluosilicate to both males and females when the M.L.D.'s are compared. The present field trials show that these three poisons rank the same in comparative value as under laboratory conditions.

CALCIUM ARSENATE

This dust ranked third in effectiveness to both males and females. Heavy applications produced a 42.2 per cent mortality in a 96-hour period in males and a 26.3 per cent mortality in females. Light applications killed 16.5 per cent of the males and 10.9 per cent of the females in the same period of time.

Out of a total of 83 males hand picked from the foliage which was heavily dusted, as they were preparing to leave, 84.1 per cent were dead in 96 hours. With the 90 females collected in this manner there was a mortality of 76.8 per cent in the same period of time.

In laboratory trials calcium arsenate did not prove to be very toxic and an M.L.D. value was not determined (Andre and Pratt, 1936). The laboratory sample used, however, was one that had been analyzed and kept in a loosely stoppered bottle since 1931, whereas all four poisons used in the field were freshly opened packages of commercial insecticides.

SODIUM FLUOSILICATE

Under field conditions this compound was least effective of the four tried. As is pointed out previously in this paper, it does not repel the beetles as much as do the other three compounds and for that reason is not included in table 5. Under laboratory conditions it was much less effective than were paris green and acid lead arsenate, a point which was also demonstrated in these field tests. A heavy application of this compound killed 25.7 per cent, whereas a light application killed 14.8 per cent of the males. In the case of the females, 12.2 per cent were killed by the heavy and 9.5 per cent by the light application.

CONTROLS

The beetles used as checks in all these tests were picked from trees not treated with any poison. By reference to table 3 it is evident that 8.4 per cent of the males and 6.0 per cent of the females died by the end of the 96-hour interval. The checks used in table 5 were the same as those used for table 4. Table 4 shows that 7.8 per cent of the males and 5.5 per cent of the females which were used as controls died in this experiment.

PERCENTAGE OF BEETLES NOT FEEDING

While studying the toxicity of paris green, acid lead arsenate, sodium fluoride and certain other insecticides under the conditions of the laboratory, it soon became evident that not all the beetles offered the various poisons would feed on them. As the writer felt that under field conditions this would be a rather important factor, a series of tests with the beetles confined individually in stender dishes were conducted to establish the percentage of adults normally refusing to eat these dusts. All these experiments, as well as the field trials, were conducted during the month of June. In the laboratory experiments the poison dusts were used alone and not mixed with bentonite.

Two per cent of the females and none of the males offered untreated leaves in the control cages refused to feed. Of those offered leaves treated with paris green 36.8 per cent of the females and 43.1 per cent of the males refused to eat. Where leaves treated with acid lead arsenate were offered the beetles, a total of 28.1 per cent of the females and 35.6 per cent of the males refused to eat. In the case of sodium fluoride, a compound not tried under field conditions, 41.2 per cent of the females and 51.2 per cent of the male beetles did not feed. Each of these feeding trials is based on 200 beetles.

It should be emphasized here that these above-mentioned figures were established when using *Phyllophaga implicita* during June and the poison dusts were used without bentonite. The writer is of the opinion that these percentages would vary—perhaps greatly under certain conditions as a number of complex interwoven factors are tied up here. Among them are the following: (1) Species of Phyllophaga being used as the test insect, (2) time of the season, (3) weather conditions at the time and immediately preceding the experiments, (4) hunger of the beetles, (5) sexual maturity of the beetles, and other external and internal conditions that one is unable to control in field experiments. One could therefore expect to get different results at different times of the year and by using different species of beetles.

SUMMARY

1. A survey was made during the summer of 1935 in an effort to determine the distribution of the various species of Brood A, June beetles in Iowa.

2. Sixty-nine counties were visited and a total of 42,559 specimens comprising 26 species of beetles were collected.

3. *Phyllophaga implicita* occurred more abundantly than any other form, while *P. hirticula* ranked second and *P. rugosa* third.

4. Field experiments with four poison dusts were conducted, namely: paris green, acid deal arsenate, calcium arsenate, and sodium fluosilicate.

Each was diluted with bentonite to form a bulk mixture of 60 per cent bentonite and 40 per cent poison dust. Under field conditions their toxicity value ranked in the descending order named.

5. A large number of beetles fed for various periods of time and left the plants when the foliage was dusted with the first three poisons mentioned. When these were collected by hand picking just before they left, a larger kill was obtained than was the case where the beetles fed on the poisoned foliage for a four-hour period. This was attributed to the fact that the beetles received a toxic dose of poison, became sick, and started to fly back to their hiding places in the soil.

6. Results obtained with paris green, acid lead arsenate and sodium fluosilicate under field conditions closely paralleled those obtained with the same compounds under laboratory conditions.

REFERENCES CITED

ANDRE, FLOYD, AND PHILIP E. PRATT
1936. The toxicity of certain stomach poisons to the June-beetle, *Phyllophaga implicita* (Horn). Iowa State College Jour. Sci., 10:243-248.

DAVIS, J. J.
1918. Common white grubs. U. S. Dept. Agric., Farmer's Bul. 940:1-30.

FLUKE, C. L.
1933. In facts for farmers. Wics. Agr. Exp. Sta., Bul. 425-85-95.

JAQUES, H. E.
1922. Brood A of the May-beetles extends its range in Iowa. Iowa Acad. Sci. Proc., 29:163-164.

———
1925. The 1924 outbreak of Brood A of white grubs in Iowa. *Ibid.* 32:423-424.

———
1926. A preliminary survey of May-beetles (Phyllophaga spp.) in Iowa. *Ibid.* 33:337-339.

———
1927. A further report on the May-beetles (Phyllophaga spp.) in Iowa. *Ibid.* 34:314-315.

———
1928. The distribution of white grubs in 1927. *Ibid.* 35:303-304.

TRAVIS, B. V.
1933. Notes on the habits of June-beetles in Iowa. (Phyllophaga-Coleoptera). Iowa State College Jour. Sci., 7:397-406.

——— AND G. C. DECKER
1933. A study on the use of arsenical dust for the control of June-beetles. *Ibid.* 7:493-498.

———
1934. Phyllophaga of Iowa. *Ibid.* 8:313-363.

VICKERY, R. A., AND T. S. WILSON
1919. Observations on Wingless May-beetles. Jour. Econ. Ent., 12:238-247.

A FLAVOR CONSTITUENT OF BLUE CHEESE (ROQUEFORT TYPE)[1]

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A desirable flavor developed in a dairy product through fermentation is ordinarily due to a combination of chemical compounds. The compounds giving a product its characteristic flavor are often present in such small amounts that their separation and identification are difficult. The problem is especially complicated with cheeses since different lots of a given variety may show considerable variation in flavor when all of them are reasonably satisfactory.

The cheeses in which *Penicillium roqueforti* or a closely related species is a normal ripening agent have a peculiar peppery flavor that is rather characteristic of this general type of product. The origin of this flavor has been considered by various investigators. In 1914, Currie (1) reviewed the early ideas along this line. From his investigations he concluded that caproic, caprylic and capric acids and their readily hydrolyzable salts have a peppery taste and are responsible for the characteristic effect of roquefort cheese on "the tongue and palate."

When the flavor of good quality blue cheese is carefully considered, with the idea of recognizing the various components, the fatty acids can ordinarily be detected but they do not appear to explain the flavor completely. The results herein reported indicate that another flavor constituent is also of importance with this type of cheese.

EXPERIMENTAL

In connection with studies on the action of *Penicillium roqueforti* on various lower fatty acids, each acid was added to sterile milk and the milk then inoculated with mold spores. Commonly there was a rapid development of mold on the surface of the milk at room temperature and volatile acid determinations on the cultures, after acidifying with sulfuric acid, indicated that the fatty acids had largely disappeared. With larger amounts of fatty acids the mold growth was greatly delayed.

A flask to which was added 600 ml. of milk, 0.3 ml. n-caprylic acid, and mold spores was of special interest since after several days at room temperature it showed no mold growth at the surface but had an odor suggesting the peppery odor of blue cheese. Later, mold growth developed and the odor disappeared. This general result was regularly obtained in trials with n-caprylic acid while trials with n-butyric, n-caproic and n-capric acids, using various concentrations of the acids, did not yield

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[2] The authors are indebted to Dr. Henry Gilman and Mr. Miles R. McCorkle for much assistance in the studies reported. Mr. McCorkle prepared the various derivatives of the unknown and known ketones and largely established the identity of the two.

the odor. Different strains of *P. roqueforti* produced the odor with caprylic acid but not with the other three.

The peppery odor developed on adding caprylic acid (0.3 ml.) and mold spores to diluted milk (600 ml.) when there was as little as 1 part of milk to 63 parts of water, but was not detected with 1 part of milk to 127 parts of water or with water alone. The odor was especially conspicuous when 1 part of milk was used with 5 parts of water since it developed rapidly under these conditions and the odor of the medium was less noticeable than when undiluted milk was employed. The addition of caprylic acid and spores to a solution of 3 gm. of dipotassium hydrogen phosphate per 600 ml. of water also yielded the odor after an incubation period of about 2 weeks; with this medium mold growth did not develop at the surface and the odor increased in intensity over an extended period.

When medium in which the peppery odor had been developed was steam distilled, the odor soon disappeared from the material in the distillation flask. Extraction with ether removed the odor from the distillate. After drying the ether extract with anhydrous sodium sulfate, it was allowed to evaporate and a small amount of liquid obtained. The peppery odor was very conspicuous in this liquid. The relationship to the odor in the original flask was most evident when a drop of the liquid was shaken with many times its volume of water; in such material the odor persisted for weeks at room temperature. Larger volumes of the liquid were prepared by distilling 5000 ml. portions of medium in which the odor had been developed by adding caprylic acid in the usual proportion, together with mold spores, and combining the distillates. Preparations from milk commonly had the odor of caprylic acid along with the peppery odor while a preparation from the phosphate medium that had been incubated for several weeks did not.

Since the peppery odor suggested an ester, a number of esters of caprylic acid were prepared. These included ethyl, n-propyl, n-butyl, n-amyl, and n-octyl caprylates. None of them had an odor resembling the peppery odor. Attention was next directed to the methyl ketones since certain of these have a conspicuous odor and the material obtained by ether extraction gave a ketone reaction. Various methyl ketones were obtained and on the basis of odor it appeared that the compound of interest was methyl-n-amyl ketone. Identification was then established as follows:

Trial 1. The liquid having the peppery odor was obtained from 20,000 ml. of diluted milk culture by steam distillation, extraction with ether, drying, and evaporation of the ether. The liquid (1.5 ml.) was distilled; after removal of a small amount of ether the temperature rose to 148° and about 1 ml. (Fraction 1) distilled between 148° and 150°. The temperature then went up rapidly and between 200° and 220° the remainder of the liquid distilled; this material had the odor of caprylic acid and was largely soluble in sodium carbonate solution. The 2,4-dinitrophenylhydrazone of Fraction 1 melted at 91-92° after a single crystallization from alcohol. It did not depress the melting point (92-93°) of the 2,4-dinitrophenylhydrazone of a known sample of methyl-n-amyl ketone.

Trial 2. A larger sample of the liquid (9 ml.) was obtained from 20,000 ml. of potassium hydrogen phosphate culture with the usual procedure. This was distilled; after the removal of the residual ether the temperature rose rapidly to 144° and about 4 ml. of the liquid came over at 146-148°. The boiling point of the known methyl-n-amyl ketone was also 146-148° with

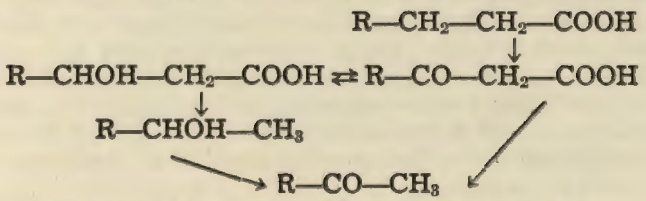
the apparatus used. The specific gravities of the unknown and known materials were 0.818 at 25°, the indexes of refraction were 1.4039 and 1.4036, respectively, at 25° and the semicarbazones melted at 121-122° alone or mixed.

Without any information as to the source of the liquids obtained from caprylic acid through the action of mold, various persons familiar with cheese described them as having the odor of blue cheese. This description was particularly convincing when given by someone especially interested in this type of cheese, either from the standpoint of consuming or marketing it.

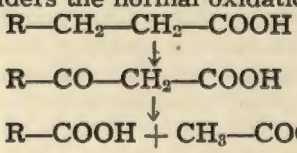
No odor suggestive of methyl-n-amyl ketone was obtained from heptylic acid through its addition to diluted milk containing spores of *P. roqueforti* but pelargonic acid yielded an odor suggesting this compound somewhat although it was still definitely different. In the original trials capric acid, which was added to the diluted milk in a liquid state, immediately solidified in relatively large masses (MP 31°). Additional trials were carried out in which the acid was added to the diluted milk, the milk warmed, and finally shaken during the cooling period to keep the acid finely divided. Under the action of the mold the capric acid yielded a compound with an odor somewhat suggestive of methyl-n-amyl ketone but still distinctly different. This odor was also slowly produced by adding the capric acid in the usual way and incubating at 37° rather than at room temperature.

The mold in the milk (diluted or undiluted) cultures yielding methyl-n-amyl ketone failed to develop in the normal manner. For a considerable period there would be no evidence of mold growth and when growth finally developed on the surface the odor of the ketone rapidly disappeared. In the phosphate medium masses of mycelium could be seen without any tendency to form a surface growth and one of the advantages of this medium was the persistence of the odor that developed.

The production of methyl-n-amyl ketone from caprylic acid suggests a beta oxidation together with the elimination of carbon dioxide from the carboxyl group. This type of change has been studied in connection with various products and presumably there are several possibilities from the standpoint of the exact steps involved. Stokoe (3) explains the formation of methyl ketones from fatty acids by molds as follows*:



He considers the normal oxidation of fatty acids to be



* The formation of the ketone from the keto acid would appear to be the more probable scheme because beta keto acids lose carbon dioxide so readily.

Stokoe believes methyl ketone is formed because the absorption of the poisonous fatty acids by the mold mycelium impedes respiration.

In a study of the methyl ketones in the oxidative decomposition of certain triglycerides and fatty acids from the standpoint of rancidity of coconut fat, Stärkle (2) considered the possibility of these compounds being important in dairy products, particularly in the rancidity of butter and in roquefort cheese. He concluded that the characteristic aroma materials in the ripening of cheese by molds are, in the case of roquefort cheese, methyl ketones instead of esters. Stärkle distilled roquefort cheese and obtained material (about 2 drops) that had an intensive odor of methyl-amyl and methyl-heptyl ketones. The mixed semicarbazone had a crude melting point of 105-107°. The amount was too small to permit separation and identification of the components. It was saponified with sulfuric acid and the odor of methyl-amyl and methyl-heptyl ketones noted.

GENERAL CONSIDERATIONS

The odor of methyl-n-amyl ketone can be detected in many lots of fine blue cheese and this compound appears to be an important flavor contributant. Along with this odor is the odor of various fatty acids, especially those of the volatile acids above butyric. In general, a conspicuous flavor of butyric acid is not as pleasing to most consumers as the flavor of the higher acids. Certain lots of cheese of satisfactory quality do not have an evident odor of the ketone although it may be present to some extent; in such cheese the desirable flavor appears to be supplied largely by the fatty acids.

The results obtained indicate that *P. roqueforti* can produce methyl-n-amyl ketone from caprylic acid. In blue cheese this acid evidently is freed from the fat by the lipase of milk or that produced by the mold. Since the mold can use the various lower fatty acids, it is probable that during the early stages of the ripening caprylic acid, together with the other volatile fatty acids, is destroyed as rapidly as formed. Later, conditions become less favorable for the normal action of the organisms, due to the lack of air, the diffusion of the salt to the interior of the cheese, the presence of the products of growth, etc., and the fatty acids accumulate. Some of the caprylic acid can be changed to methyl-n-amyl ketone and some of the capric acid may be changed to methyl-n-heptyl ketone; the latter transformation is suggested by the work of Stärkle (2). On the basis of the odor of blue cheese, methyl-n-amyl ketone would appear to be the important ketone involved. In the trials reported, the odor of methyl-n-amyl ketone disappeared from the cultures when active growth of the molds began so it is probable that a destruction of this compound also occurs in blue cheese. In cheese that does not have the odor of the ketone, the compound may have been destroyed or conditions may never have been satisfactory for its formation.

The butter defect which is commonly described as "roquefort flavor" very definitely suggests the odor of methyl-n-amyl ketone and undoubtedly this compound can be formed in butter through the action of molds under unfavorable growth conditions. Fat hydrolysis would yield caprylic acid and this could be changed to the ketone in quite the same way as in milk cultures or in blue cheese.

CONCLUSIONS

Methyl-n-amyl ketone is an important flavor contributant of blue cheese. The odor is conspicuous in many lots of cheese but is not evident in others although it may be present to some extent. This compound apparently is formed from caprylic acid through the action of *P. roqueforti* under unfavorable growth conditions.

LITERATURE CITED

1. CURRIE, JAMES N.
1914. Flavor of roquefort cheese. Jr. Agr. Res., 2:1.

2. STÄRKLE, MAX
1924. Die Methylketone im oxydativen Abbau einiger Triglyzeride (bzw. Fettsäuren) durch Schimmelpilze unter Berücksichtigung der besonderen Ranzidität des Kokosfettes. Biochem. Ztschr. 151:371.

3. STOKOE, WILLIAM NORMAN
1928. The rancidity of coconut oil produced by mould action. Biochem. Jr., 22:80.

DISSIMILATION OF PYRUVIC ACID BY THE PROPIONIC ACID BACTERIA[1]

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Virtanen (1923) was the first to study the fermentation of pyruvic acid by the propionic acid bacteria; from three molecules of the fermented acid he obtained one molecule of propionic acid and two each of acetic acid and CO_2. Van Niel (1928) concluded that acetaldehyde is not an intermediate, and suggested that the pyruvic acid is hydrated at the alpha-carbon and subsequently dehydrogenated to acetic acid and CO_2. Virtanen and Karström (1931) found that dried propionic bacteria were unable to ferment pyruvic acid except in the presence of phosphate. Support of the intermediate nature of pyruvic acid was given by Wood and Werkman (1934), who isolated it from the fermentation of glucose by sulfite fixation. They were unable to detect acetaldehyde. Later Erb, Wood and Werkman (1936) found pyruvic acid as a final product in the aerobic dissimilation of lactic acid by cell suspensions of propionic bacteria. They suggested that the bacteria catalyze the following reaction:

(1) $CH_3 . CHOH . COOH \rightleftarrows CH_3 . CO . COOH + 2H$

The present investigation of the dissimilation of pyruvic acid has been made as part of a general study of the dissimilation of proposed intermediates of the propionic acid fermentation. Only by making a critical study of intermediate dissimilation can a true picture of the whole process be obtained. Schemes of dissimilation founded wholly on the final products frequently lead to erroneous conclusions.

METHODS

The experimental procedure was that described by Erb, Wood and Werkman (1936). The Barcroft-Warburg respirometer (according to Dixon, 1930) and the macro-respirometer described by Wood, Erb and Werkman (1936) were used. The pyruvic acid (Eastman) was twice distilled under reduced pressure. A dilute solution neutralized to the proper pH with NaOH was sterilized by Seitz filtration. The acid was determined by iodoform titration before addition of buffer and bacteria. Succinic acid was determined, after distillation of the volatile acids, by extraction with ether and precipitation as the silver salt. Volatile acids, CO_2, lactic acid, unfermented pyruvic acid and oxygen utilized were determined as previously described by Erb et al. (1936). The bacterial suspension was not aerated previous to use.

Results given in tables 1, 2 and 3 were obtained by the Barcroft-Warburg technic with 2 ml. of medium containing 0.25 ml. of a suspension of 1 part of wet bacterial mass and 9 parts of water, 1.5 ml. of 0.15 M phosphate buffer and 0.25 ml. of 1.6 per cent neutralized pyruvic acid.

[1] Supported in part by Industrial Science Research funds of Iowa State College.

TABLE 1. *Carbon dioxide production and oxygen utilization in the aerobic dissimilation of pyruvic acid at different pH values**

Part I

Time in hours	Initial pH	Endogenous O_2	Endogenous CO_2	Pyruvic acid O_2	Pyruvic acid CO_2	Net totals O_2	Net totals CO_2	Ratio CO_2: O_2	Final pH
		mm³	mm³	mm³	mm³	mm³	mm³		
8	5.20	281.6	219.4	546.6	701.7	265.0	482.3	1.8	5.6
8	5.55	291.1	233.1	507.5	641.9	216.4	408.8	1.9	5.75
8	6.20	337.7	220.2	386.4	424.8	48.7	204.6	4.2	6.45
8	6.90	374.1	254.1	388.0	341.7	13.9	87.6	6.3	6.85

* By *P. arabinosum* (34 W) Age of cells = 72 hours + 37 hours in icebox.

Part II

8	5.85	183.2	178.1	300.7	440.4	117.5	262.3	2.2	5.50
8	6.30	179.0	156.3	268.9	374.0	89.9	217.7	2.4	6.00
8	6.70	176.4	155.6	263.8	313.6	87.4	158.0	1.8	6.35

* By *P. pentosaceum* (49 W) Age of cells = 72 hours + 8 hours in icebox.

In the macro-respirometer studies, five grams of wet bacterial mass were suspended in 500 ml. of medium consisting of 0.15 M. phosphate buffer at pH 5.6 and 0.7 per cent neutralized pyruvic acid (pH 5.6). Endogenous respiration values have been subtracted. Oxygen and CO_2 were the only endogenous values of quantitative significance. On a basis comparable to quantities given in table 4, the endogenous oxygen-uptake varied from 15 to 19 mM and the CO_2 from 19.3 to 23.2 mM. The anaerobic endogenous CO_2 was 7.9 mM. The time of incubation was 68 hours in experiments 1 and 2 and 32 hours in 3 and 4.

EXPERIMENTAL

The optimal pH range for the dissimilation of pyruvic acid was first determined (tables 1 and 2). The lowest pH used (5.2) in the aerobic

TABLE 2. *Carbon dioxide production in the anaerobic dissimilation of pyruvic acid at different pH values**

Time in hours	Initial pH	Endogenous CO_2 mm³	Pyruvic acid CO_2 mm³	Net totals CO_2 mm³	Final pH
8	5.25	64.6	174.4	109.8	5.15
8	5.65	45.8	571.8	526.0	5.50
8	6.00	57.7	402.4	344.7	5.60
8	6.25	42.5	238.6	196.1	6.10
8	6.80	31.9	166.9	135.0	6.50
8	7.50	41.6	75.3	33.7	7.00

* By *P. pentosaceum* (49 W) Age of cells = 72 hours + 8 hours in icebox.

experiments gave the largest oxygen-uptake as well as CO_2 production. Unpublished results show that the O_2 uptake and CO_2 evolved markedly decrease below pH 5.0. Table 2 shows the maximum CO_2 produced in the anaerobic dissimilation to be approximately pH 5.5. The same pH range was found optimal for lactic acid dissimilation (Erb et al. 1936). It is of interest that change in pH definitely affects the dissimilation, as shown by the variation in the CO_2: O_2 ratio, particularly with *Propionibacterium arabinosum* (table 1). Increase in pH resulted in an increase in the ratio. This effect is the opposite of that obtained with lactic acid in which the ratio decreased with increase in pH.

The oxygen uptake and the CO_2 production per hour (table 3) show a relative change as indicated by shifting of the ratio of CO_2: O_2. During the initial stage of dissimilation the ratio is high and then decreases. The converson of pyruvic acid to acetic acid and CO_2 requires one atom of oxygen (equation 2) and the ratio CO_2: O_2 equals 2.

$$(2)\quad CH_3 . CO . COOH + H_2O \rightarrow CH_3 . C(OH)_2 . COOH \xrightarrow{+O} CH_3COOH + CO_2 + H_2O$$

TABLE 3. *Carbon dioxide production and oxygen utilization in the aerobic dissimilation of pyruvic acid*

Initial pH 5.55; final pH 5.75

P. arabinosum (34 W)*

Time in hours	Endogenous O_2	Endogenous CO_2	Pyruvic acid O_2	Pyruvic acid CO_2	Net rate per hr. O_2	Net rate per hr. CO_2	Net totals O_2	Net totals CO_2	Ratio CO_2: O_2
	mm³	mm³	mm³	mm³	mm³	mm³	mm³	mm³	
1	74.1	46.3	99.6	123.2	25.5	76.9	25.5	76.9	3.0
2	46.8	32.9	54.5	100.4	7.7	67.5	33.2	144.4	4.3
3	54.5	38.6	78.2	89.7	23.6	51.1	56.8	195.5	3.4
4	36.4	34.3	70.7	82.9	34.3	48.6	91.1	244.1	2.6
5	24.0	25.3	62.6	70.1	38.6	44.8	129.7	288.9	2.2
6	20.1	19.1	56.2	65.8	36.1	46.7	165.8	335.6	2.0
7	16.2	18.4	46.3	61.2	30.1	42.8	195.9	378.4	1.9
8	18.8	18.2	39.4	48.5	20.6	30.3	216.5	408.7	1.8

Initial pH 5.85; final pH 5.50

P. pentosaceum (49 W)**

1	41.6	37.7	47.5	75.1	5.9	37.4	5.9	37.4	6.3
2	35.7	35.5	42.9	65.0	7.2	29.5	13.1	66.9	5.1
3	21.4	20.5	37.7	57.3	16.3	36.8	29.4	103.7	3.5
4	20.8	20.4	37.7	53.1	16.9	32.7	46.3	136.4	2.9
5	19.5	17.7	35.9	51.7	16.4	34.0	62.7	170.4	2.7
6	11.7	15.1	30.7	47.6	19.0	32.5	81.7	202.9	2.4
7	17.5	15.5	35.3	46.3	17.8	30.8	99.5	233.7	2.3
8	14.9	15.7	33.0	44.2	18.1	28.5	117.6	262.2	2.2

* Age of cells = 72 hours + 37 hours in icebox.
** Age of cells = 72 hours + 8 hours in icebox.

A ratio greater than 2 indicates that some additional substance other than O_2 is functioning as a hydrogen acceptor. A number of investigators have shown that the propionic acid bacteria do not form acetaldehyde and for this reason the decarboxylation of pyruvic acid to CO_2 and acetaldehyde is not considered. It seems probable that the high ratio is caused by part of the pyruvic acid acting as a hydrogen acceptor, in part replacing O_2, to form lactic acid (reaction 1). Since this oxidation of pyruvic acid-hydrate to acetic acid and CO_2 involves no utilization of oxygen, a high ratio occurs. Inasmuch as propionic acid is the normal reduction product of anaerobic fermentation of pyruvic acid by propionic acid bacteria, it seems probable that the lactic acid is reduced in turn to propionic acid. On the other hand, the decrease in the ratio of CO_2: O_2 to approximately 2 indicates that the net results of the final conversion may have been that shown in equation 2, i. e., a quantitative oxidation of the pyruvic acid to acetic acid and CO_2. If this is the case, it also involved oxidation of any propionic and lactic acids which may have been formed to acetic acid and CO_2. The oxidation of lactic acid appeared possible but a similar oxidation of propionic acid did not seem probable since propionic acid usually has been considered a stable end-product of the fermentation. This indicated that propionic acid was not produced and that only lactic acid occurred which was formed in establishment of an equilibrium between pyruvic and lactic acids (Eq. 1). Furthermore, after equilibrium is reached, the oxygen acts as the hydrogen acceptor. This function of O_2 as an acceptor will bring about a decrease in the ratio of CO_2: O_2 and as an increasing amount of pyruvic acid is dissimilated the ratio will approach 2.

TABLE 4. *Dissimilation of pyruvic acid by Propionibacterium arabinosum (34 W)*

Number	Pyruvic acid fermented per liter	Products per 100 mM of fermented pyruvic acid						O_2 utilized per 100 mM of fermented pyruvic acid	Carbon recovered	Redox index*
		Total volatile acid	Propionic acid	Acetic acid	CO_2	Lactic acid	Succinic acid			
	mM	mM	mM	mM	mM	mM	mM	mM	Pctg.	
1	82.4	90.9	17.2	73.7	98.5	1.0	0.0	46.1	100.1	0.94
2	81.1	92.5	11.1	81.4	95.4	0.6	0.0	38.6	94.5	1.01
3	83.4	95.0	26.3	68.7	66.7	0.0	1.6	4.0	96.5	1.00
4	73.5	97.8	36.3	61.5	58.0	0.7	0.1	anaerobic	97.4	0.85

* Cf. Erb, Wood and Werkman. J. Bact. 13:595 (1936).

A perfect balance is indicated by an index $\frac{\text{Oxidized}}{\text{Reduced}} = 1.0$. The O_2 utilized is assumed to be reduced to water and has, therefore, a reduction value of 2.0. Since the pyruvic acid is not neutral with respect to oxidation — reduction, as glucose is, but must be reduced by one molecule of hydrogen to be neutral, it is assigned a reduction value of 1 when used as a substrate.

In an endeavor to prove whether or not lactic acid occurs and to obtain more complete information concerning the mechanism of pyruvic acid dissimilation quantitative determination of the products was made with the macro-respirometer (table 4). The results show that there was no accumulation of lactic acid. Only final analyses are given in the table but experiments were run simultaneously and under similar conditions in which the lactic acid was determined at short intervals. In no case was there sufficient accumulation of lactic acid under either aerobic or anaerobic conditions to be of quantitative significance. This indicates that there may have been an oxidation of propionic acid; therefore the dissimilation of propionic acid was investigated. It was found that this acid as well as acetic acid is dissimilated in the presence of air with a utilization of O_2 and a producton of CO_2. These results will be reported in a separate communication. It is evident that aerobic dissimilation by propionic acid bacteria may result in a complete oxidation to CO_2. The ratio of CO_2: O_2 is not determined alone by the initial conversion of the pyruvic acid but also by the subsequent dissimilation of the propionic and acetic acids.

The results in table 4 give a more complete picture of the changes. The determinations are reasonably accurate inasmuch as the carbon and oxidation-reduction balances are satisfactory with the exception of fermentation 4 in which the redox index suggests an error.

Fermentation 3 though conducted in the presence of air was predominantly an anaerobic dissimilation for little oxygen was utilized. In this experiment it was noted that the flask was not being shaken as vigorously as in fermentations 1 and 2 and the medium probably was not saturated with air. Fermentations 1 and 2 which utilized considerable oxygen, show an increased yield of acetic acid and CO_2 and a decrease of propionic acid.

The mechanism of aerobic and anaerobic dissimilation in the initial breakdown of the pyruvic acid are probably the same, involving pyruvic acid-hydrate (equation 2). Under anaerobic conditions part of the pyruvic acid functions as a hydrogen acceptor to be reduced to propionic acid while aerobically O_2 replaces the pyruvic acid as an acceptor, diminishing the propionic acid. Under weakly aerobic or anaerobic conditions (fermentations 3 and 4) equivalent quantities of acetic acid and CO_2 were formed but with strong aeration, the oxidation is more vigorous as shown by the production of CO_2 in greater quantities than the acetic acid. At present any explanation of the mechanism of this phase of the oxidation must be speculative. Evidence (Wood and Werkman, 1936[1, 2]) indicates that propionic bacteria can synthesize succinic acid from acetic acid. Succinic acid is decomposed by resting cells with formation of CO_2 (unpublished data). The increased production of CO_2 under strongly aerobic conditions may result from a condensation of acetic acid and decarboxylation and oxidation of the condensation product.

SUMMARY

The dissimilation of pyruvic acid by cell suspensions of propionic acid bacteria has been shown to be most active at approximately pH 5.5. Propionic acid, acetic acid and CO_2 constitute the principal products. Acetic acid and CO_2 are probably formed by hydration and dehydrogenation of the pyruvic acid at the alpha-carbon, propionic acid probably by reduc-

tion of pyruvic acid through lactic acid. In aerobic dissimilation oxygen replaces the pyruvic acid as a hydrogen acceptor and there is a decreased yield of propionic acid. The mM of CO_2 were found equal to the mM of acetic acid under anaerobic or weakly aerobic conditions, but on strong aeration the mM of CO_2 were greater than the acetic acid. The increased CO_2 may result from oxidation of succinic acid which originates by condensation of two molecules of acetic acid.

REFERENCES

DIXON, M.
1930. Manometric methods. Cambridge University Press.

ERB, CARL, H. G. WOOD AND C. H. WERKMAN
1936. J. Bact., 31:595-602.

VAN NIEL, C. B.
1928. The propionic acid bacteria. Haarlem.

VIRTANEN, A. I.
1923. Soc. Sc. Fennica Comment. physic.-math., 1 No. 36, 1-23.

VIRTANEN, A. I., AND H. KARSTRÖM
1931. Acta Chem. Fennica, series B, 7:17-19.

WOOD, H. G., AND C. H. WERKMAN
1934. Biochem. J., 28:745-747.

———————, C. ERB AND C. H. WERKMAN
1936. Iowa State College J. Sc., 10:295-302.

——————— AND C. H. WERKMAN
1936. Biochem. J., 30:48-53.

——————— ———————
1936. Biochem. J., 30:618-623.

THE EFFECT OF PHOSPHATE FERTILIZERS ON THE REACTION OF GRUNDY SILT LOAM IN GREENHOUSE EXPERIMENTS[1]

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The effect of fertilizers on the reaction of the soil has long attracted the attention of many investigators. Such materials as sodium nitrate and calcium cyanamide are known to leave basic residues, whereas, ammonium sulfate and potassium chloride have been found to leave acid residues in the soil. The continued use of any of these materials over a long period of years has been shown to influence considerably the reaction of the soil. The results of studies on the effect of phosphate fertilizers on the reaction of soils, however, have been quite variable. A neutralizing value for superphosphate has been reported (8) (13) (22). On the other hand some investigators (17) (24) (27) have found an increase in acidity following its use, while others (10) (15) (16) noted no change in reaction.

In some cases rock phosphate has appeared to have a neutralizing effect on acid soils (8) (27) (28) while in other tests no effect was noted. The latter conclusion was reached by a number of investigators (7) (19) (25) (26).

The claim has been made that rock phosphate can take the place of lime in acid soils. Rock phosphate may supply calcium as a plant nutrient and it may neutralize some of the soil acidity. Because of the difficulty of separating the nutritional effects of calcium-bearing materials from their neutralizing powers, investigators have been doubtful as to the relative importance of the two functions. Among those who believe that much of the benefit of calcium-bearing materials on plants growing in acid soils is due to the calcium available for plant nutrition are Robinson and Williams (20), Albrecht (1) (2), Whitson, Chapman and Hull (30) and some others (3) (5) recognize the importance of the nutritive value of calcium.

Pierre (19) found that the original reaction of the soil influenced the effect of different phosphate fertilizers on soil reaction as measured by pH over a period of 18 months. The more acid the soil, the greater was the neutralizing effect of the fertilizer. The different effects of the phosphates on soils varying in acidity were explained as due possibly to the different ways in which phosphate fixation occurs at different levels of acidity. He used superphosphate, rock phosphate, tricalcium phosphate, dicalcium phosphate, monocalcium phosphate and monosodium phosphate on three soils, one of high acidity, one medium, and one low. All the phosphates used reduced the acidity of the very acid soil. The neutralizing effects were less marked on the soil of medium acidity, monocalcium phosphate not affecting the reaction and superphosphate even increasing the acidity. On the slightly acid soil the neutralizing effects of tricalcium and

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TABLE 1. *The effect of rock phosphate and sodium phosphate alone and with lime and of lime alone on the pH of Grundy silt loam*

Treatment	pH													Treatment means
	1933									1934				
	1–11	2–18	3–11	4–11	5–19	7–11	8–14	9–14	11–23	1–13	3–10	4–11	8–11	
1. Check	5.24	5.18	5.28	5.04	5.04	5.03	4.98	4.73	4.99	5.03	5.00	4.69	4.93	
	5.24	5.18	5.28	5.10	5.04	5.03	5.01	4.84	4.99	5.06	5.00	4.71	5.00	5.02
2. 500 lbs. rock phosphate	5.32	5.23	5.36	5.09	5.17	5.01	5.01	4.85	5.02	4.98	5.11	4.69	4.93	
	5.27	5.23	5.33	5.10	5.17	5.03	4.98	4.88	5.02	5.00	5.11	4.69	4.96	5.06
3. 1000 lbs. rock phosphate	5.29	5.23	5.31	5.12	5.14	5.06	4.98	4.85	5.02	5.03	5.09	4.66	4.95	
	5.32	5.23	5.28	5.15	5.14	5.06	5.01	4.83	5.02	5.01	5.09	4.69	4.90	5.06
4. 1500 lbs. rock phosphate	5.34	5.18	5.28	5.15	5.11	5.03	4.98	4.81	4.97	5.01	5.06	4.61	4.91	
	5.34	5.18	5.28	5.15	5.14	5.02	5.01	4.90	4.97	5.01	5.07	4.66	5.27	5.06
5. 2000 lbs. rock phosphate	5.34	5.20	5.28	5.12	5.14	5.06	5.01	4.88	5.06	5.05	5.07	4.68	5.00	
	5.32	5.23	5.24	5.09	5.14	5.05	4.98	4.89	5.07	5.08	5.11	4.71	4.96	5.07
6. 2500 lbs. rock phosphate	5.34	5.22	5.28	5.12	5.24	5.15	4.94	4.88	5.04	5.08	5.02	4.69	4.90	
	5.34	5.24	5.24	5.15	5.24	5.13	4.96	4.95	5.06	5.15	5.08	4.75	4.98	5.08
7. 3000 lbs. rock phosphate	5.31	5.10	5.36	5.15	5.18	5.08	4.98	4.95	5.09	5.02	5.03	4.71	4.93	
	5.34	5.14	5.38	5.17	5.18	5.08	4.98	4.99	4.99	5.02	5.07	4.71	4.86	5.07
8. 1000 lbs. rock phos. + 4 T. lime	7.36	7.13	7.12	6.82	6.75	6.62	6.59	6.45	6.45	6.55	6.51	5.47	6.42	
	7.38	7.08	7.09	6.80	6.75	6.62	6.55	6.44	6.47	6.55	6.51	5.50	6.33	6.63
9. 2000 lbs. rock phos. + 4 T. lime	7.46	7.09	7.18	6.95	6.78	6.69	6.72	6.60	6.57	6.55	6.51	5.60	6.43	
	7.49	7.11	7.19	6.92	6.75	6.65	6.66	6.60	6.60	6.62	6.51	5.60	6.37	6.70
10. Na_3PO_4 = 1000 lbs. rock phos.	5.60	5.48	5.56	5.59	5.27	5.15	5.15	5.11	5.14	5.09	5.17	5.05	5.13	
	5.65	5.48	5.59	5.59	5.31	5.16	5.15	5.19	5.14	5.07	5.17	5.07	5.20	5.28
11. Na_3PO_4 = 2000 lbs. rock phos.	5.93	5.85	6.02	5.61	5.54	5.41	5.38	5.30	5.30	5.31	5.32	5.01	5.23	
	5.92	5.84	6.04	5.66	5.54	5.38	5.38	5.32	5.35	5.34	5.32	4.95	5.23	5.48
12. Na_3PO_4 = 1000 lbs. rock phos. + 4 T. lime	7.64	7.39	7.46	7.02	7.03	6.77	6.73	6.59	6.62	6.69	6.68	5.58	6.53	
	7.65	7.41	7.49	7.04	7.06	6.75	6.75	6.56	6.61	6.69	6.67	5.47	6.53	6.82
13. Na_3PO_4 = 2000 lbs. rock phos. + 4 T. lime	7.71	7.44	7.66	7.12	7.21	6.91	6.82	6.69	6.79	6.78	6.69	6.08	6.60	
	7.71	7.44	7.66	7.12	7.21	6.93	6.82	6.68	6.81	6.78	6.71	5.88	6.53	6.95
14. 4 T. lime	7.50	7.24	7.22	7.02	6.91	6.62	6.61	6.59	6.61	6.60	6.50	5.83	6.30	
	7.47	7.26	7.18	7.02	6.93	6.62	6.60	6.54	6.54	6.60	6.50	5.70	6.30	6.72

Average of pH of soil before treatment was 5.31.

dicalcium phosphate were very slight, whereas, monocalcium phosphate caused a slight increase in acidity and superphosphate an appreciable increase.

Cook and Connor (9) found considerable differences in the effect of rock phosphate and c.p. tricalcium phosphate upon the acidity developed by fertilizers when added to two soils, depending upon the method employed to determine the reaction. In one soil the two phosphates reduced the acidity measured by the Jones and Hopkins methods but when measured by percentage saturation and pH, the acidity was increased except in one case. In the other soil the tricalcium phosphate had no effect except when the Hopkins method was used and in this case the greatly reduced lime requirement was attributed to the fixation of aluminum by the phosphate.

EXPERIMENTAL

The primary aim of this experiment was to study the effect of rock phosphate on soil reaction and to attempt to determine whether or not rock phosphate could be substituted for lime by supplying the calcium required in plant nutrition. Rock phosphate was selected because of its use in a finely ground form as a fertilizer known as lime phosphate (14) and because of recommendations that rock phosphate be used in mixed fertilizers to correct some of the acidity present in them or resulting from their use (23).

An attempt was made to isolate any effects of the calcium supplied by the rock phosphate from its neutralizing effect. If some of the benefit from liming is due to the available calcium supplied, it is possible that rock phosphate may be substituted for lime by supplying calcium to the plant. In order to obtain some information on this point sodium phosphate was substituted for rock phosphate in several cases.

METHODS OF PROCEDURE

The pH measurements were made with the quinhydrone electrode according to the recommendations of Biilman and Jensen (6). The Hardy and Lewis (11) lime requirement method was used. In the base exchange studies, measurements of exchangeable calcium and determinations of exchangeable hydrogen were made by the ammonium acetate method of Schollenberger and Dreibelbis (21).

In measuring base exchange capacity a modification of the procedure recommended by Schollenberger and Dreibelbis was followed. An open system so arranged as to keep the soil covered with the solution and to provide a continuous flow was developed and found to give comparable results with the closed system. Results obtained by this method compared closely with those obtained by Parker's (18) method.

Available phosphorus was determined by the 0.002 N sulfuric acid method of Truog (29). Harper's (12) modification of the phenoldisulfonic acid method was used to determine the nitrates. The official method (4) was followed in determining the calcium content of sweet clover, the magnesium nitrate method was used for total soil phosphorus and the Gunning-Hibbard method for total nitrogen.

The effects of different amounts of rock phosphate and of sodium phosphate in comparison with limestone on the reaction of Grundy silt

TABLE 2. *The effect of rock phosphate and sodium phosphate alone and with lime and of lime alone on Grundy silt loam in the greenhouse*

Treatment*	Mean pH **	Mean lime requirement, lbs. per acre ***	Exch. H. M.E. per 100 gms. soil ****	Avail. PO_4 p.p.m. *****	NO_3-N p.p.m. +	Exch. Ca M.E. per 100 gms. soil NH_4Ac. method ++
1. Check	5.02	6211	9.56	32.20	153.8	14.76
2. 500 lbs. rock phos.	5.06	6014	10.10	55.92	167.7	14.88
3. 1000 lbs. rock phos.	5.06	5989	11.38	82.50	168.0	14.78
4. 1500 lbs. rock phos.	5.06	6119	10.60	115.34	150.6	15.54
5. 2000 lbs. rock phos.	5.07	5782	10.59	142.36	156.3	16.04
6. 2500 lbs. rock phos.	5.08	5608	9.60	168.67	155.3	15.52
7. 3000 lbs. rock phos.	5.07	5616	11.55	194.03	161.9	16.03
8. 1000 lbs. rock phos. and 4 T. lime	6.63	1384	5.23	96.10	288.3	22.77
9. 2000 lbs. rock phos. and 4 T. lime	6.70	1463	5.81	147.16	277.3	23.11
10. Na_3PO_4 = 1000 lbs. rock phosphate	5.28	5212	10.32	69.58	154.0	15.15
11. Na_3PO_4 = 2000 lbs. rock phosphate	5.48	4955	8.68	102.37	181.8	15.74
12. Na_3PO_4 = 1000 lbs. rock phos. and 4 T. lime	6.82	1238	2.67	89.20	299.6	23.77
13. Na_3PO_4 = 2000 lbs. rock phos. and 4 T. lime	6.95	1172	2.05	132.37	310.8	23.22
14. 4 T. lime	6.72	1192	2.53	41.83	314.0	23.31
Highly significant difference (P = .01)	.02+	90				
Significant difference (P = .05)		71				

*—Each treatment in duplicate pots.
**—Average of 13 samplings over 20 month period.
***—Average of 9 samplings over 20 month period.
****—Average of 3 samplings over 20 month period.
*****—Average of 3 samplings over 12 month period.
+—One sampling after 16 month period.
++—Average of 3 samplings over 20 month period.

TABLE 3. *Analysis of variance of pH values of Grundy silt loam treated with different amounts of rock phosphate and sodium phosphate with and without lime and with lime alone*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	363	265.1973	.7306
Within date—treatment groups	182	.1907	.001
Between means of dates	12	26.99	2.25**
Between means of treatments	13	229.67	17.67**
Interaction	156	8.35	.05

** Means highly significant.

TABLE 4. *Influence of different amounts of rock phosphate and sodium phosphate alone and in combination with lime and of lime alone on the lime requirement of Grundy silt loam in pounds per acre*

Treatment-	Date of sampling									Treatment mean
	1-11 1933	2-18 1933	3-11 1933	4-11 1933	5-19 1933	7-11 1933	7-11 1933	8-14 1933	3-10 1934	
Check	6206	5280	6570	7884	6189	5931	5889	5995	5959	6211
500 lbs. rock phosphate	6111	5071	6074	6945	6214	6042	5830	5913	5924	6014
1000 lbs. rock phosphate	6111	4888	6104	6926	5796	6398	5749	5971	5960	5989
1500 lbs. rock phosphate	6165	4630	6078	7663	5821	6828	5789	5889	6216	6119
2000 lbs. rock phosphate	4142	4703	6115	6705	6214	6729	5667	5796	5971	5782
2500 lbs. rock phosphate	6098	4396	4470	5784	5772	6361	5633	5936	6030	5608
3000 lbs. rock phosphate	5989	4433	4630	5868	6018	6361	5655	5690	5901	5616
1000 lbs. rock phosphate + 4 T. lime	910	884	1350	1548	2002	1596	1519	1028	1624	1384
2000 lbs. rock phosphate + 4 T. lime	815	1253	1130	1597	1817	1818	1730	1531	1484	1463
Na_3PO_4 = 1000 lbs. rock phos.	5018	4077	3684	4323	6079	6312	6006	5691	5726	5212
Na_3PO_4 = 2000 lbs. rock phos.	4970	3905	4617	2997	6361	5465	5200	5399	5691	4955
Na_3PO_4 = 1000 lbs. rock phos. + 4 T. lime	856	1130	1191	811	1400	1548	1473	1344	1391	1238
Na_3PO_4 = 2000 lbs. phos. + 4 T. lime	978	1130	713	841	1424	1572	1496	1075	1321	1172
4 T. lime	964	848	762	958	1535	1609	1531	1192	1332	1192

Least mean difference highly significant = 90

TABLE 5. *Analysis of variance of lime requirement of Grundy silt loam treated with different amounts of phosphate fertilizers with and without lime and treated with lime alone*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	251	1,258,911,091	5,015,584*
Within date—treatment groups	126	1,450,749	11,514*
Between means of dates	8	33,932,390	4,241,549*
Between means of treatments	13	1,163,997,898	89,538,300*
Interaction	104	59,530,654	572,410*

* Highly significant.

TABLE 6. *Analysis of variance of lime requirement of Grundy silt loam treated with different amounts of rock phosphate (same data as used in table 4 but omitting the results for the soils treated with lime or with sodium phosphate)*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between means of dates	8	34,140,566	4,267,571*
Between means of treatments	6	6,212,110	1,035,352*
Interaction	48	19,749,713	411,452*

* Highly significant.

TABLE 7. *Exchangeable hydrogen content and total exchange capacity of Grundy silt loam by the ammonium-acetate method and the calculated amount of exchangeable bases and the degree of saturation*

Treatment	7-11-33 (7 months)				11-23-33 (11 months)				8-11-34 (20 months)				Treatment means for exchange Hydrogen M.E.
	Exch. H. M.E.	Exch. capacity M.E.	Exch. bases M.E.	Degree of saturation Pctg.	Exch. H. M.E.	Exch. capacity M.E.	Exch. bases M.E.	Degree of saturation Pctg.	Exch. H. M.E.	Exch. capacity M.E.	Exch. bases M.E.	Degree of saturation Pctg.	
1. A Check	8.81	27.58	18.77	68.0	10.01	27.58	17.57	63.7	10.25	27.58	17.33	62.8	9.56
B	8.20		19.38	70.2	9.84		17.74	64.3	10.25		17.33	62.8	
2. A 500 lbs. rock	9.64		17.94	65.0	10.15		17.43	63.2	10.25		17.33	62.8	10.10
B phosphate	9.64		17.94	65.0	10.25		17.33	62.8	10.66		16.92	61.3	
3. A 1000 lbs. rock	10.87		16.71	60.5	10.25		17.33	62.8	12.92		14.66	53.2	11.38
B phosphate	10.25		17.33	62.8	10.25		17.33	62.8	13.74		13.84	50.2	
4. A 1500 lbs. rock	9.23		18.35	66.5	9.64		17.94	65.0	12.92		14.66	53.2	10.60
B phosphate	9.23		18.35	66.5	.....		.....	.....	12.92		14.66	53.2	
5. A 2000 lbs. rock	9.23		18.35	66.5	10.25		17.33	62.8	13.74		13.84	50.2	10.59
B phosphate	8.61		18.97	68.8	8.20		19.38	70.3	13.53		14.05	50.9	
6. A 2500 lbs. rock	8.00		19.58	71.0	6.15		21.43	77.7	14.35		13.23	48.0	9.60
B phosphate	7.79		19.79	71.7	6.97		20.61	74.7	14.35		13.23	48.0	
7. A 3000 lbs. rock	7.79		19.79	71.7	11.89		15.69	56.9	14.76		12.82	46.4	11.55
B phosphate	7.38		20.20	73.2	12.71		14.87	53.9	14.76		12.82	46.4	
8. A 1000 lbs. rock	1.23		26.35	95.5	8.81		18.77	68.1	4.10		23.48	85.1	5.23
B phos. + 4 T. lime	1.03		26.55	96.3	9.84		17.74	64.3	6.36		21.22	76.9	
9. A 2000 lbs. rock	1.03		26.55	96.3	7.59		19.99	72.5	6.77		20.81	75.4	5.81
B phos. + 4 T. lime	2.46		25.12	91.1	10.25		17.33	62.8	.....		.....	.....	
10. A Na_3PO_4 = 1000	7.18		20.40	74.0	12.51		15.07	54.6	10.87		16.71	60.5	10.32
B lbs. rock phos.	6.77		20.81	75.4	12.71		14.87	53.9	11.89		15.69	56.9	
12. A Na_3PO_4 = 2000 lbs.	5.95		21.63	78.4	11.89		15.69	56.9	.....		.....	.....	8.68
B rock phos.	5.13		22.45	81.4	12.71		14.87	53.9	8.20		19.38	70.3	
12. A Na_3PO_4 = 1000 lbs.	0		27.58	100.0	3.08		24.50	88.8	2.87		24.71	89.6	2.67
B rock + 4 T. lime	1.03		26.55	96.3	6.15		21.43	77.7	2.87		24.71	89.6	
13. A Na_3PO_4 = 2000 lbs.	1.64		25.94	94.1	3.08		24.50	88.8	1.44		26.14	94.8	2.05
B rock + 4 T. lime	0.82		26.76	97.0	2.87		24.71	89.6	2.46		25.12	91.1	
14. A 4 T. lime	1.64		25.94	94.0	2.87		24.71	89.1	2.05		25.53	92.6	2.53
B	1.03		26.55	96.3	4.10		23.48	85.1	3.49		24.09	87.3	

Note: The values of base exchange capacity, exchangeable hydrogen and exchangeable bases are expressed in terms of milli-

loam were studied in the experiment. Grundy silt loam, having a lime requirement of approximately 3½ tons per acre and a pH of 5.30, was brought to the greenhouse, sieved through a ¼-inch screen and 30 pounds of dry soil placed in each of fifty-six 4-gallon pots. The average phosphorus content of this original soil was 0.08 per cent and the average nitrogen content was 0.23 per cent.

The soils were treated according to the plan shown in table 1. The moisture content of the soils was adjusted to 50 per cent of the saturation capacity and maintained at that amount by additions of distilled water. Of the four pots per treatment, two were fallowed and used for sampling and two were seeded to sweet clover. A sub-irrigation system was used in watering the cropped pots. The fallow soils were sampled at intervals for determinations of pH, exchangeable hydrogen and base exchange capacity, exchangeable calcium, available phosphorus, nitrate nitrogen, and lime requirement.

RESULTS

pH DETERMINATIONS

The pH determinations were made over a 20-month period (table 1) and the treatment means are given in table 2. Wherever lime was used the pH was markedly increased. However, sodium phosphate with lime was more effective in raising the pH than was lime alone, whereas, rock phosphate with lime did not increase in every case where rock phosphate was used. The differences between the treatment means are of more consequence than if they were those of individual comparisons, yet it is doubtful if the differences are large enough to actually show a treatment effect. In order to test the significance of the variability resulting from these treatments an analysis of variance was made of the data. The total variability due to treatment was found to be highly significant (table 3).

LIME REQUIREMENT

Lime requirement data for the variously treated soils using samples taken at nine different dates are shown in table 4 and the treatment means are given in the second column of table 2. Lime and sodium phosphate each decreased the lime requirement of Grundy silt loam and there also seemed to be some tendency for rock phosphate to decrease the lime requirement of this soil. An analysis of variance (table 5) for all 14 treatments showed that there was a highly significant variability between treatments. This was also true of the variability resulting from the rock phosphate treatments alone (table 6). These results agree in general with those showing the pH. The higher amounts of rock phosphate generally produced lower lime requirements. Sodium phosphate was more effective than equivalent amounts of rock phosphate. The higher rate of sodium phosphate with lime was as effective as lime alone, actually more effective numerically, but only by a negligible difference. This was not true of the lower rate of sodium phosphate. Rock phosphate with lime was not as effective as lime alone.

Cook and Conner (9) attributed an increase in acidity following phosphate applications to nitrification. Since this might be the reason why rock phosphate with lime was not as efficient as lime alone, the nitrate

nitrogen content of the soil was determined. In table 2 it appears that no more nitrate was present where phosphates were used with lime than where lime was used alone.

BASE EXCHANGE PROPERTIES

Base exchange capacity, exchangeable hydrogen content, content of exchangeable bases and degree of saturation are given in table 7. An analysis of the data for base exchange capacity showed no significant differences which could be attributed to treatment. This would justify the use of the mean value for base exchange capacity which was 27.58 M.E. per 100 gms. of soil. Since the base exchange capacity did not vary with treatment, the base exchange content when figured as the difference between base exchange capacity and replaceable hydrogen, will vary inversely as does the hydrogen content. Hence only the results for exchangeable hydrogen are given in table 2. The marked effect of lime in lowering the content of exchangeable hydrogen is apparent. Sodium phosphate also decreased the exchangeable hydrogen content appreciably, but rock phosphate did not produce significant differences (table 8). As was the case when measured by pH and by the Hardy and Lewis lime requirement method, the higher rate of sodium phosphate used with lime was again found to be the most effective treatment.

Large increases in exchangeable calcium were found in all the limed soils by the ammonium acetate method (table 9) but an analysis of the data (table 10) revealed no significant differences between the means of the exchangeable calcium contents of the soils treated with rock phosphate alone.

GROWTH, YIELD AND CALCIUM CONTENT OF SWEET CLOVER AND AVAILABLE PHOSPHORUS AND NITRATE-NITROGEN CONTENT OF THE SOIL

In the early stages of growth there was a noticeably greater growth and greener color of sweet clover where rock phosphate was used than on the untreated soils. There was some tendency for the growth to be more vigorous as the rates of application increased. In contrast to all the other treatments a very poor stand and stunted growth of clover were obtained on soils treated with applications of sodium phosphate alone and with the lime alone. Sodium phosphate with lime and rock phosphate with lime produced only a fair growth which was not as good as that on some of the soils receiving higher rates of rock phosphate alone. Later

TABLE 8. *Analysis of variance of exchangeable hydrogen content of Grundy silt loam treated with different amounts of rock phosphate*

Source of variation	D.f.	Total sum of squares	Mean square
Total	41	208.82	
Within	21	3.92	0.187
Between means of dates	2	118.92	59.46**
Between means of treatment	6	22.45	3.74
Interaction	12	63.53	5.29**

** Mean square highly significant.

all the soils receiving some form of phosphate supported sweet clover crops of about equal vigor, whereas, the untreated soils produced poor crops, quite obviously below the average of the phosphate treated soils. However, differences in color were quite noticeable. The sweet clover in one of the untreated soils was light green, but the clover in the other one was normal. Clover in all the soils receiving different amounts of rock phosphate either alone or in combination with lime showed no chlorosis. The clover in the other treated soils including those receiving different amounts of sodium phosphate either alone or with lime and the soils receiving lime alone all showed distinct chlorosis. A few weeks later practically all the chlorosis had disappeared.

The first cutting of hay was made on June 11 and the second on August 5. The average dry weights of these cuttings are given in table 11. A statistical analysis of these data as presented in table 12 indicates that the average yields with the various treatments varied significantly.

The rock phosphate treated soils, except the 1500 pound per acre

TABLE 9. *Exchangeable calcium content of Grundy silt loam by the ammonium-acetate method of Schollenberger*

Treatment		M.E. Exch. Ca. per 100 gms. soil			
		Date			
		7-11-33	11-33-33	8-11-34	Treatment means
1.	A Check	15.30	16.74	12.92	
	B	15.30	16.23	12.09	14.76
2.	A 500 lbs. rock phos.	17.57	15.09	12.71	
	B	15.71	15.51	12.71	14.88
3.	A 1000 lbs. rock phos.	16.54	15.30	12.82	
	B	16.12	15.09	12.82	14.78
4.	A 1500 lbs. rock phos.	16.95	15.71	14.68	
	B	15.71	15.50	14.68	15.54
5.	A 1000 lbs, rock phos.	16.33	17.36	15.09	
	B	15.71	16.74	14.99	16.04
6.	A 2500 lbs. rock phos.	15.30	16.95	14.78	
	B	15.30	15.71	15.09	15.52
7.	A 3000 lbs. rock phos.	15.30	15.71	14.99	
	B	15.71	16.12	13.33	16.03
8.	A 1000 lbs. rock phos. + 4 T. lime	23.15	22.12	23.36	
	B	22.74	21.91	23.36	22.77
9.	A 2000 lbs. rock phos. + 4 T. lime	24.39	22.94	23.15	
	B	23.56	21.70	22.94	23.11
10.	A Na_3PO_4 = 1000 lbs. rock phos.	14.88	15.63	14.68	
	B	15.09	15.63	14.99	15.15
11.	A Na_3PO_4 = 2000 lbs. rock phos.	14.88	16.74	14.88	
	B	15.71	17.16	15.09	15.74
12.	A Na_3PO_4 = 1000 lbs. rock phos.	23.25	25.01	22.84	
	B + 4 T. lime	23.77	23.15	24.49	23.77
13.	A Na_3PO_4 = 2000 lbs. rock phos.	23.36	23.36	23.15	
	B + 4 T. lime	23.36	23.15	22.94	23.22
14.	A 4 T. lime	23.36	23.98	22.84	
	B	23.15	23.05	23.46	23.31

TABLE 10. *Analysis of variance in exchangeable calcium content of Grundy silt loam treated with different amounts of rock phosphate*

Source of variation	D.f.	Total sum of squares	Mean square
Total	41	71.91	
Within	21	5.95	0.283
Between means of dates	2	41.77	20.885*
Between means of treatment	6	8.22	1.37
Interaction	12	15.97	1.33

* Mean square highly significant.

treatment, produced highly significant increases in yield. Although some variability occurred in the results from the 500, 1000, 1500, and 2000 pound rates, rates of 2500 and 3000 pounds per acre produced mean yields greater than any of the other treatments by highly significant amounts. Likewise, the 2000 pound rate caused a significant increase in mean yield above all the lesser rates except the 1000 pounds per acre. All the treated soils yielded more sweet clover than the untreated soil. Hence,

TABLE 11. *The yield of sweet clover and the calcium content of sweet clover on Grundy silt loam*

Treatment	Yield in gms. ave. of 1st & 2nd cuttings	Pctg. Ca in Sw. Cl. ave. of 1st & 2nd cuttings
1. Check	18.55	2.212
2. 500 lbs. rock phosphate	22.47**	2.096
3. 1000 lbs. rock phosphate	19.17	2.180
4. 1500 lbs. rock phosphate	20.47**	2.170
5. 2000 lbs. rock phosphate	22.93**	2.088
6. 2500 lbs. rock phosphate	24.97**	2.291
7. 3000 lbs. rock phosphate	24.95**	2.026
8. 1000 lbs. rock phosphate + 4 T. lime	25.72*	2.392
9. 2000 lbs. rock phosphate + 4 T. lime	26.68*	2.362
10. Na_3PO_4 = 1000 lbs. rock phosphate	26.51	1.998
11. Na_3PO_4 = 2000 lbs. rock phosphate	26.84	2.014
12. Na_3PO_4 = 1000 lbs. rock phosphate + 4 T. lime	28.53**	2.282
13. Na_3PO_4 = 2000 lbs. rock phosphate + 4 T. lime	31.79**	2.328
14. 4 T. lime	28.87	2.438
Highly significant difference (P = .01)	3.83	
Significant difference (P = .05)	2.53	

* Significantly greater than the yield of any treatment above it in column.
** Highly significantly greater than the yield of any treatment above it.

it may be said that applications of rock phosphate increased the yield of sweet clover in most cases to a highly significant extent and that there was a tendency for this increase to be proportional to the amount of rock phosphate added.

The soils receiving lime or sodium phosphate produced mean yields of sweet clover significantly greater than that on the untreated soils. Rock phosphate with lime increased the yield over that from equivalent amounts of rock phosphate alone by significant amounts. One thousand and 2000 pounds of rock phosphate per acre with 4 tons of lime and the equivalent applications of sodium phosphate did not differ from each other significantly in respect to the mean yields of sweet clover.

The highest mean yield of sweet clover was produced by the higher rate of sodium phosphate in combination with lime. This yield was greater than that with either the lime alone or the lime with the lower rate of sodium phosphate by significant values and greater than the yields with any of the other treatments by highly significant amounts. All three of the last named treatments produced yields significantly greater than those resulting from any other treatment. It is interesting to note that the treatment producing the highest yield of sweet clover, namely, 4 tons of lime with sodium phosphate equivalent to 2000 pounds of rock phosphate, also produced the highest pH, the lowest lime requirement and the lowest content of exchangeable hydrogen.

DISCUSSION

Although each 500-pound application of rock phosphate did not result in a large increase in pH of the Grundy silt loam in greenhouse tests, there was a tendency for the higher amounts to be more effective (figure 1). This same tendency was even more noticeable when the reaction was measured by the Hardy and Lewis lime requirement method. Rock phosphate did not produce a significant difference in reaction of Grundy silt

TABLE 12. *Analysis of variance of sweet clover yields on Grundy silt loam*

Source of variation	Degrees of freedom	Sum of squares	Mean square
A Untreated soil and soils treated with rock phosphates only			
Total	13	424.92	
Within—treatment groups	7	99.26	14.18
Between means of treatments	6	325.65	54.88*
B Entire 14 treatments of Experiment			
Total	27	1813.0	
Within—treatment groups	14	298.8	21.34
Between means of treatments	13	1514.9	116.48**

* Significant
** Highly significant.

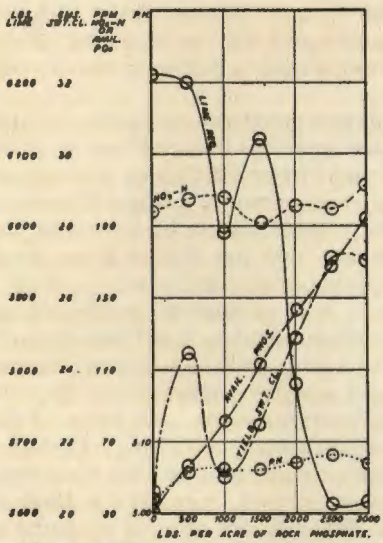

Fig. 1. Effect of rock phosphate on Grundy silt loam.

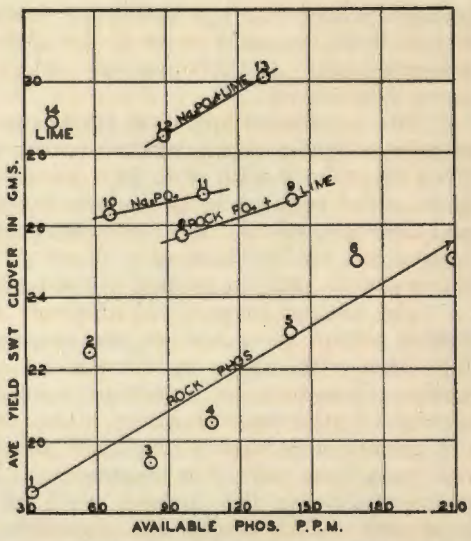

Fig. 2. The relation of available phosphorus to yield of sweet clover. Note: Numbers refer to treatment as in table 1.

loam, however, as measured by the base exchange capacity, the exchangeable hydrogen content, or the degree of saturation. Observations on the growth of clover revealed an early stimulating effect from all rates of application of rock phosphate, although there was not a great difference

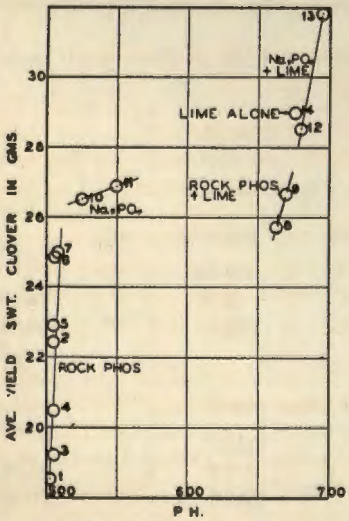

Fig. 3. The relation of pH to yield of sweet clover. Note: Numbers refer to treatment as in table 1.

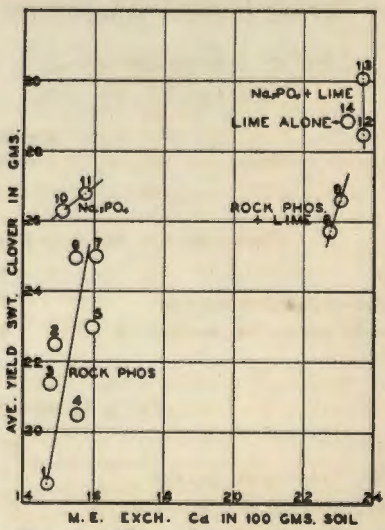

Fig. 4. The relation of exchangeable calcium to yield of sweet clover. Note: Numbers refer to treatment as in table 1.

between treatments. A significant increase in yield was obtained wherever rock phosphate was used, the two highest rates of application being associated with the highest yields. The percentage of calcium in the sweet clover plants was highest wherever lime was used. Rock phosphate produced no apparent effect on the calcium content of the sweet clover except in the case of the 2500-pound rate.

Except for 4 tons of $CaCO_3$ alone, the nutritional value of the phosphate added seemed to be an important variable affecting the growth of sweet clover on soils of similar pH and of similar exchangeable calcium content (figure 2). However, the kind of phosphate and the addition of lime with the phosphate affected the yield noticeably. The various treatments in which amounts of phosphate equivalent to that in 1000 pounds of rock phosphate were used did not show much variation in the amount of available phosphate in the soil following treatment but showed large differences in yields of sweet clover. This variability is greatly affected by type of treatment, that is, sodium phosphate with lime and lime alone produced the highest yields, sodium phosphate alone the next highest, rock phosphate with lime the next and rock phosphate alone the least of the treated soils. Lime applied with the phosphates in these tests did not depress the availability of the phosphorus.

Since the different combinations of fertilizers do not produce the same yields even when the amounts of available phosphate resulting from the treatments are approximately the same, it is apparent that other factors than the available phosphate supplied affected the yield of the sweet clover. That soil reaction is probably one of the most important of these factors may be seen in figure 3. However, the decrease in acidity produced by sodium phosphate does not seem to be large enough to account for all the effect of this material on sweet clover. The soils receiving lime and rock phosphate had much higher pH values than did the soils receiving only sodium phosphate but the yields of sweet clover were not noticeably different.

The effect of exchangeable calcium on yield is shown in figure 4. Why the very large increases in content of exchangeable calcium and in pH associated with the limed soils as compared with the soils receiving sodium phosphate alone did not cause greater increases in yield than they did is not known. The high figures for exchangeable calcium may be due to calcium dissolved from calcium carbonate by the reagents used. The rather small increases in yield with large increases in pH following liming may be due to the interaction between pH and other variables affecting yield. Large amounts of soluble phosphorus or calcium in the soil may augment the effect of pH on yield. In the case of the sodium phosphate treated soils the sodium may cause the increased yield of sweet clover previously pointed out.

A multiple correlation was made of the pH, the p.p.m. of available phosphorus, the p.p.m. of nitrate nitrogen, the exchangeable H and exchangeable calcium content of the soil, the percentage calcium in the crop and the yield of sweet clover in grams. The results (table 13) show that the amount of available phosphorus in the soil was not significantly correlated with yields of sweet clover. This does not prove, however, that the beneficial effect of rock phosphate on sweet clover was due to the lime supplied by the rock phosphate. This type of correlation is obviously due to the results produced by lime, a material which stimulated the growth of sweet clover greatly but added no phosphate.

A study of table 13 shows the high interrelation of the different factors to each other and emphasizes the difficulty of determining which of them was responsible for the effects of the different treatments. The very marked increase in both exchangeable calcium and pH and equally marked decrease in exchangeable hydrogen following applications of lime probably accounts for a large degree of the correlation between these variables.

When pH was isolated from the other variables it still had a correlation to yield that was highly significant. This was not true of the other factors studied.

The β values in table 13 show that pH was the most important independent variable affecting yield. Exchangeable calcium is next in importance, exchangeable hydrogen next and the effects of available phosphorus and nitrate nitrogen are negligible. In view of the very large effects produced by lime, these correlations doubtless show the effects of lime more than they do those of rock phosphate. Nevertheless, the same trends may be expected to hold true to a certain extent where rock phosphate is used alone.

SUMMARY AND CONCLUSIONS

Studies were made of the effect of finely-ground rock phosphate and sodium phosphate, each applied alone and in combination with lime and of lime alone on the reaction of Grundy silt loam in the greenhouse under uniform conditions. Changes in reaction were measured periodically by the quinhydrone electrode method, the Hardy and Lewis lime require-

TABLE 13. *The correlation of pH, exchangeable hydrogen, exchangeable calcium, nitrate nitrogen and available phosphorus content of Grundy silt loam and of the yield and calcium content of sweet clover*

	Nitrate-nitrogen	Exch. H.	Exch. Ca	Avail. P.	Yield Sw. Cl.	Pctg. Ca in Sw. Cl.
pH	.9880**	—.9610**	.9852**	—.0566	.8632**	.7454**
Nitrate N.		—.9654**	.9824**	—.1000	.8006**	.7816**
Exch. H			.9410**	.1674	—.8149**	—.7603**
Exch. Ca				.0107	.7736**	.7839**
Avail. P.					—.0774	—.1045
Yield Sw. Cl.						.3173

** Highly significant correlation.

.63 Least significant correlation factor.

.66 Least highly significant correlation factor.

β Values

	Beta	r
β yield x Avail. P.	= +.0303	—.0774
β yield x % Ca in Sw. Cl.	= —.6892	+.3173
β yield x Exch. H.	= +.5136	—.8149
β yield x Nitrate N.	= +.1684	+.8006
β yield x Exch. Ca	= —.5667	+.7736
β yield x pH	= +1.2550	+.8362

ment method and by determining base exchange properties. In an attempt to isolate and evaluate the importance of the changes in reaction produced by the different treatments the variously treated soils were analyzed for content of exchangeable calcium, available phosphorus and nitrate nitrogen, and the effect of the different treatments on the yield and the calcium content of two crops of sweet clover was determined. The results of these studies may be summarized as follows:

1. Rock phosphate, sodium phosphate and lime did not cause significant changes in the base exchange capacity of Grundy silt loam.

2. Applications of 500 pounds per acre or more of finely ground rock phosphate produced slight decreases in the acidity of Grundy silt loam as measured by pH, and by lime requirement (Hardy and Lewis).

3. The small neutralizing effects of finely ground rock phosphate showed a tendency to increase with increased rates of application.

4. Finely ground rock phosphate caused highly significant increases in the yield of sweet clover on Grundy silt loam and there was a tendency for increased rates of application to produce higher yields.

5. There was a highly significant correlation between the reaction of Grundy silt loam and the yield of sweet clover. Likewise, the treatments producing higher pH values consistently produced higher yields regardless of the amount of available phosphorus present in the soil.

6. There was evidence that decreasing the acidity of Grundy silt loam as measured by pH, lime requirement and exchangeable hydrogen was more important in increasing the yield of sweet clover than were the changes produced in the content of replaceable calcium, nitrate nitrogen, or available phosphorus in the soil.

7. Sodium phosphate was more efficient in neutralizing acidity of Grundy silt loam than was rock phosphate applied in equivalent amounts on the basis of P_2O_5 content.

8. No evidence was found showing that rock phosphate could substitute for lime by supplying calcium to the plant. However, a high content of exchangeable calcium in the soil and a high percentage of calcium in the plant were associated with the highest yields of sweet clover. Yet where phosphate fertilizers were added there was no measurable increase in these constituents. Further studies on the effect of phosphate fertilizers on the soluble calcium content of the soil are desirable.

9. Four tons per acre of calcium carbonate did not noticeably depress the amount of soluble phosphorus in the soil.

LITERATURE CITED

1. ALBRECHT, WM. A.
1932. Calcium and hydrogen-ion concentration in the growth and inoculation of soy beans. Jour. Amer. Soc. Agron., 24:793-806.

2. ———
1933. Inoculation of legumes as related to soil acidity. Jour. Amer. Soc. Agron., 25:512-522.

3. ——— AND FRANKLIN L. DAVIS
1929. Relation of calcium to the nodulation of soy beans on acid and neutral soils. Soil Sci., 28:261-280.

4. Association of Official Agricultural Chemists
1930. Official and tentative methods of analysis. Third Edition. Association of Official Agricultural Chemists, Washington, D. C.

5. Bauer, F. C.
1923. The foraging power of plants for phosphate rock. Jour. Amer. Soc. Agron., 15:99-109.

6. Billman, E., and S. T. Jensen
1927. On the determination of the reaction of soils by means of the quinhydrone electrode. Trans. 2nd Comm. Int. Soc. Soil Sci., 1926, B:236-274.

7. Brown, P. E.
1932. The mystery of the soil. Jour. Amer. Soc. Agron., 24:935-950.

8. Conner, S. D.
1916. Acid soils and the effect of acid phosphate and other fertilizers upon them. Jour. Ind. Eng. Chem., 8:35-41.

9. Cook, H. L., and S. D. Conner
1936. A study of the basicity of dolomite, rock phosphate, and other materials in preparing non-acid forming fertilizers. Jour. Amer. Soc. Agron., 28:843-855.

10. Hance, F. E.
1932. Report on Chemistry. Proc. Ann. Meeting Hawaiian Sugar Planters' Assoc., 51:619-642. (Abst. in Chem. Abst., 26:5685. 1932; original not seen.)

11. Hardy, F., and A. H. Lewis
1929. A rapid electrometric method for measuring lime requirement of soils. Jour. Agr. Sci., 19:17-25.

12. Harper, H. J.
1924. The accurate determination of nitrates in soils. Jour. Ind. Engr. Chem., 16:180-183.

13. Harrison, C. J.
1932. The acidity of tea soil of northeast India. III. The effect of fertilizers on soil acidity. Indian Tea Assoc., Sci. Dept. Quart. Jour., 1932:189-199. (Abst. in Chem. Abst., 28:2824, 1934; original not seen.)

14. Hoover, R. P.
1929. Radio talk over Station WENR. Mimeographed sheet by Ruhm Phosphate and Chem. Co.

15. Jensen, S. Tovbory
1933. Commercial fertilizers and soil reaction. Proc. Intern. Congr. Soil Sci., 2:154-161. (In English.)

16. Joret, Georges
1934. Are superphosphate and plaster capable of lowering the pH of the soil. Engrais 49:119. (Abst. in Chem. Abst., 28:2828, 1934; original not seen.)

17. Lauder, A., and Alex M. Smith
1934. The effect of various fertilizers on soil acidity. Agr. Progress 11:93-98.

18. Parker, F. W.
1929. 3. The determinations of exchangeable hydrogen in soils. Jour. Amer. Soc. Agron., 21:1030-1039.

19. Pierre, W. H.
1934. The effect of mono-, di-, and tricalcium phosphates on the reaction of soils of different degrees of acidity. Jour. Amer. Soc. Agron., 26:278-289.

20. Robinson, G. W., and Rice Williams
1924. Base exchange in relation to the problem of soil acidity. Trans. Faraday Soc., 20:586-593.

21. SCHOLLENBERGER, C. J., AND F. R. DREIBELBIS
1930. Analytical methods in base exchange investigations on soils. Soil Sci., 30:161-173.

22. SEWELL, M. C., AND W. L. LATSHAW
1931. The effect of lime, superphosphate, and potash on reaction of soil and growth and composition of alfalfa. Jour. Amer. Soc. Agron., 23:709-814.

23. SHUEY, P. McG.
1934. Ground phosphate rock as a substitute for filler in fertilizer mixtures. The Amer. Fert. 80, 10:8, 9, 26.

24. SKINNER, J. J., AND J. H. BEATTIE
1916. Influence of fertilizers and soil amendments on soil acidity. Jour. Amer. Soc. Agron., 9:25-35.

25. SMITH, F. B., P. E. BROWN, J. B. PETERSON AND F. E. SCHLOTS
1932. The influence of lime and phosphate fertilizers on soil reaction. Jour. Amer. Soc. Agron., 24:469-476.

26. SMITH, R. L.
1935. Phosphate rock as filler substitute in fertilizer mixtures. Com. Fertilizer, 51:14, 16, 18, 20. Nov.

27. SNIDER, H. J.
1934. A chemical study of a soil under long-continued field experiments. Jour. Amer. Soc. Agron., 26:946-953.

28. THOR, A. U.
1933. The availability and downward movement of rock phosphate in Illinois soils when used liberally for 25 to 30 years and influence of the treatment on available potash and total nitrogen. Jour. Amer. Soc. Agron., 25:661-674.

29. TRUOG, E.
1930. Proposed method for readily available phosphorus of soils. Jour. Amer. Soc. Agron., 22:874-882.

30. WHITSON, A. R., H. D. CHAPMAN AND H. H. HULL
1929. Not soil acidity, but lack of calcium, which limits alfalfa culture. Wisc. Agr. Exp. Sta. Rep. for 1927-1928:105-106. Wisc. Agr. Exp. Sta. Bul. 405.

BIOLOGICAL ASSAY OF FEEDING STUFFS IN A BASAL RATION FOR COCCIDIUM-GROWTH-PROMOTING SUBSTANCE

I. PROCEDURE, YELLOW CORN MEAL, OATS, OAT HULLS, WHEAT, LINSEED MEAL, MEAT SCRAP[1]

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The general methods employed in the biological assay of food materials for vitamin content are familiar to the biologist and biological chemist. After it had been demonstrated (Becker and Morehouse, 1937) that a number of food materials contain a thermostable (to autoclaving) principle which favors the development of coccidium *Eimeria nieschulzi* (Synonym: *E. miyairii*) in its rat host and that one food stuff (such as powdered yeast) may contain more of it than another (such as dry powdered pork liver), it seemed feasible to try to adapt the biological assay method to testing the ordinary feeding stuffs in a standard basal ration for their relative contents of this coccidium-growth stimulant, which elsewhere we have designated "coccidibios".

Owing to a number of difficulties, such as immunity considerations, lack of uniformity in vitality of cultures of the parasite, and variability in individual host susceptibility, it was obviously not feasible to attempt to determine units of coccidibios on the basis of the number of oöcysts of the parasite eliminated per gram of the tested material fed, in a manner comparable to the practice of estimating vitamin B or vitamin G units from the number of grams of body weight gained by a young animal per gram of a material fed as the exclusive source of the vitamin. A more promising alternative appeared to be determining a ratio between the number of oöcysts obtained when infected hosts were on one diet and the number obtained when hosts were on a standard diet, the requisite condition being, of course, that the test and reference series of experiments are carried on simultaneously and all other conditions of the experiment are as nearly alike for the two series as possible. The entire procedure finally adopted in order to derive an estimation of the relative coccidium-growth-promoting properties of feeding stuffs involves a number of important considerations, and will be explained in detail.

PLAN OF PROCEDURE

The Microörganism. The parasite employed throughout was *Eimeria nieschulzi* Dieben, which in former papers had been referred to as *Eimeria miyairii* (*Cf.* Roudabush, 1937). It parasitizes the gland cells of the small intestine of the wild brown rat and the tame variety. The particular strain was one obtained originally from a wild rat and carried on in white

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rats for several years, then inbred for four successive generations by infecting previously uninfected rats with single oöcysts. The oöcysts to be "cultured" were obtained from the caeca of heavily infected rats on the ninth day of the infection and washed three times by centrifugation. The last residue containing myriads of oöcysts was then mixed with 2 per cent potassium dichromate solution and poured into Petri dishes for the three-day sporulation period. The sporulated or infective stages were kept in an electric refrigerator. In order to maintain infective microörganisms with a high degree of vitality, the cultures were renewed about once a month.

The Host. The host employed throughout was the highly inbred Wistar A strain of albino rat. Every animal used had been raised in the laboratory from previously immunized females, and had remained uninfected from birth to the time of experimental infection. Ordinarily the average weight of the litter was between 60 gm. and 80 gm. when experimental feeding commenced. Previously mother and young had received a growing ration (Steenbock's) made up by pounds of the following materials: yellow corn meal, 76; linseed meal, 16; commercial casein, 5; ground alfalfa, 2; salt, 0.5; ground oyster shell, 0.5. Fresh milk was given every other day *ad libitum.* One or more litters were divided as evenly as possible according to sex and weight in order to obtain test and reference series for a particular trial.

The Diets. The reference or control diet in all cases consisted by parts of the following: beet sugar, 72; casein (unextracted), 15; salt mixture (Hawk and Oser), 4; lard, 3; cod liver oil, 2; Fleischmann's powdered yeast, 4. Rats grow moderately well on this diet throughout the duration of the experiment. A further reason for selecting 4 per cent as the amount of dry yeast was that this amount in the diet resulted in a moderate, but not excessive, growth of the parasite in the host after the infective dose of parasites had been administered.

The test or assay diets were made up by substituting the material to be assayed for the four parts of yeast and part of the beet sugar, so that the whole added up to 100 parts. The more concentrated feeding materials containing 30 per cent or more of protein were fed at the 10 per cent level, while less concentrated materials were fed at the 30 per cent level; for example, an oats diet would consist by parts of the following: beet sugar, 46; casein, 15; salt mixture, 4; lard, 3; codliver oil, 2; ground whole oats, 30.

When the young rats had attained the proper weight they were placed on the test and control diets for 9 or 10 days previous to the date of the first experimental infection. During this time and subsequently they received only the special ration and water.

Infection. As previously stated, the first infective dose of oöcysts occurred at the end of the ninth or tenth day on the special diets, and another on the twelfth or thirteenth day. The doses of 2,000 organisms were administered directly into the stomach through a catheter while the rat was under ether.

Collection of Oöcysts. When the rats were infected as described, elimination of oöcysts commenced 7 days after the first infective dose, and practically ceased after 6 or 7 days. During this period of oöcyst elimination the rats were kept in individual cages floored with half-inch mesh hardware cloth and suspended over a flat pan holding 0.5 per cent "Kresol"

solution. The fecal pellets dropped directly from the rat into the disinfectant that prevented putrefaction.

Counting Oöcysts. The plan was to determine the number of oöcysts eliminated by test and reference rats during the entire infection, not the number per gram of feces. Two counts were made for each rat, one at the end of the third day of the collection period and the other after oöcyst elimination had practically ceased.

The pellets were disintegrated as thoroughly as possible in the collection pans by means of a masher made of a rubber stopper with a solid glass rod inserted into the smaller end for a handle. For further disintegration and mixing, the content of the pan was poured into a tin quart measure and thoroughly agitated with an electric mixer. Then it was poured into a glass cylinder and made up to 1,000 c.c. with water. After thorough mixing in another container, a sample of the mixture was quickly poured into a glass tumbler. The counts were made by means of a haemocytometer with a chamber of 0.9 cubic millimeter capacity over the ruled area. The actual number of oöcysts in two chambers, or 1.8 cubic millimeters, was determined by counting under the low power of the microscope. If the numbers of oöcysts in the two chambers did not check within about 10 per cent, two more counts were made. On the basis of these sample counts it was easy to calculate the approximate number of oöcysts passed by a discharging animal during the collection periods. The total number of oöcysts passed by a rat during an infection was the sum of the numbers passed during the two collection periods.

Treatment of Data. The data have been treated, admittedly, in a somewhat arbitrary manner. In each test the numbers of oöcysts passed by the rats on the 4 per cent yeast or reference diet have been taken as the standard for comparison with the other diets. On this basis it has been possible to define a yeast coefficient (*F*) as follows: *F* is the ratio of the number of oöcysts passed by 10 or more rats on a test diet to the number passed by 10 or more rats on the reference diet. Of course, the experimental procedure as previously outlined must have been closely followed for the coefficient to have any value.

It is also true that the coefficient expresses the relative coccidium stimulating properties of the rations as wholes, and not simply of the constituent variables; i. e., if the *F* value for a diet made up to 30 per cent with ground wheat were 1, this value could not be claimed to express simply a comparison of the coccidigenic properties of 30 parts of wheat and 4 parts of dry yeast, for even without wheat or yeast in the ration there would have been some development of the microörganism (*V.* Becker and Morehouse, 1935).

In the tables there appears a *W*-value representing the approximate mean ratio of the gains in weight of the assay series for the first 16 or 17 days on the special diets to the same for the reference series. The 16- or 17-day period will be referred to as the growth period of record.

ASSAY OF YELLOW CORN MEAL

The yellow corn meal used in these experiments was prepared by the Beaver Valley Milling Company, Des Moines, Iowa. It was fed at the 30 per cent level in the test ration. Corn is known to contain liberal amounts of vitamins A, B and E, to be rather deficient in vitamin G, and to be al-

most totally lacking in vitamin D. The latter was supplied in the cod liver oil. Since the casein was unextracted, there was some vitamin G from this source. The experiment was conducted in three trials.

The columns in table 1 headed "Wt. Gain" show the gains in weight made over a 16- or 17-day period, beginning the first day on the special diets and ending the seventh day of the infection. There is usually no weight loss due to coccidiosis before the eighth day when the infective doses are no larger than those employed in these experiments, no matter what the diet. Weight data for the remainder of the infection are not included in the table because there is often considerable irregularity attributable to the infection. The comments in this paragraph apply also to the tests of other feeding stuffs subsequently discussed.

The table shows that both series of rats made considerable growth gains, with but slight advantage in favor of the corn-feds, for the mean ratio of the gains of the test to the reference series (*W*-values) was 1.092.

The ratio of the number of cysts eliminated by the corn-fed series to the number eliminated by the yeast-fed series, or *F*-value, was .474. The logical deduction is that the basal ration made up with 30 parts of yellow corn meal has less than half the coccidium-growth-promoting properties of the same made up instead with 4 parts of Fleischmann's powdered yeast.

TABLE 1. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent yellow corn meal diets*

	(1) Reference series		(2) Test series		Ratios (2): (1)	
Rat Number	Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcysts counts
1	33	250	33	48		
2	25	168	44	56		
3	30	140	42	31		
4	33	127	48	147		
5	30	129	47	54		
Mean	30.2	162.8	42.8	67.2	1.417	.413
6	55	75	62	42		
7	53	64	43	73		
8	48	79	38	17		
9	43	63	33	7		
10	56	60	32	63		
Mean	51.0	68.2	41.6	40.4	0.816	.592
11	28	77	24	19		
12	36	142	29	64		
13	21	25	29	20		
14	28	62	40	12		
15	26	30	23	25		
Mean	27.8	67.2	29.0	28.0	1.043	.417
W					1.092	
F						.474

TABLE 2. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground hulled oats diets*

		(1) Reference series		(2) Test series		Ratios (2): (1)	
Trial	Rat Number	Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcyst counts
1	1	37	407	34	89		
	2	60	310	44	285		
	3	died	died	66	118		
	Mean	48.5	358.5	48.0	164.0	0.99	.457
2	4	38	165	60	22		
	5	37	112	41	113		
	6	44	124	39	18		
	Mean	39.7	133.7	46.7	51.0	1.18	.381
3	7	34	144	26	249		
	8	30	243	35	176		
	9	26	184	64	134		
	Mean	30.0	190.3	41.7	186.3	1.39	.979
4	10	23	211	39	50		
	11	22	188	40	131		
	12	26	236	28	120		
	13	25	210	33	157		
	14	33	171	36	115		
	Mean	25.8	203.2	35.2	114.6	1.36	.564
W						1.25	
F							.591

It may be questioned whether in this case the difference between the effects of the two diets is actual or explainable on the basis of probability alone. In order to make a statistical test of this point, the data were subjected to an analysis of variance test following Snedecor's (1934) solution of a problem in which more than one item was involved in the classes. An "F"-value of 16.01 was obtained, whereas the tables of "F"-values show 7.81 as the value which would not be exceeded in random sampling from a homogeneous population once in a hundred trials. Therefore, the results of the two series do differ more than could ordinarily be expected if the diets were without effect.

ASSAY OF HULLED OATS

The hulled oats were obtained from a local feed store, carefully picked over to free them from foreign grains and oat hulls, and ground moderately fine in a coffee mill. The ground oats were fed at the 30 per cent level. According to Morrison's (1936) tables, oat kernels without hulls contain about 16.2 protein. Oats is known to be a good source of vitamins B and E, but not so good for vitamin G, and to lack vitamins A and D. The experiment was conducted in four trials involving 13 rats

in the controls and 14 in the test series. The weight and oöcyst enumeration data are shown in table 2.

Both series of rats increased steadily in weight on the diets during the growth period of record, but the oat-feds outgained the controls by about a fourth when the mean gains are compared.

Here, as in the case of the yellow corn meal tests, the grain recipients passed fewer oöcysts than the reference series, for the *F*-value was .591. The third trial was considerably out of line with the others, for there was little difference between the oöcyst counts for the two series, but it is to be expected on the basis of mere probability that such an outcome would occasionally be met with in trials involving only three rats in each series, even though the series represent different populations. The fourth trial shows definitely that the oat diet, which produced the greater weight gain, resulted in a lower oöcyst production.

ASSAY OF OAT HULLS

Finely ground oat hulls were prepared by the Quaker Oats Company upon the special request of the writers, and grateful acknowledgment for the courtesy is made at this place. They were fed at the 30 per cent level, making a rather bulky mixture. The rats became accustomed to the ration, however, and each actually made a mean gain of a gram and a half a day during the growth period of record. The data are presented in table 3. There was but one trial. The *W*-value of .688 shows that the test series grew better than two-thirds as fast as the reference series. The *F*-value of .494 indicates that the oat hulls did not exert a very pronounced stimulating effect upon the reproduction of the coccidium.

TABLE 3. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground oat hull diets*

	(1) Reference series		(2) Test series			
Rat Number	Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	*W*	*F*
1	49	249	26	53		
2	42	247	36	153		
3	42	258	18	238		
4	26	237	28	132		
5	32	254	34	135		
6	36	299	16	74		
7	47	255	24	165		
8	39	296	28	114		
9	38	259	17	124		
10	31	198	36	78		
Mean	38.2	255.2	26.3	126.1		
					.688	.494

TABLE 4. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 30 per cent ground whole wheat diets*

		(1) Reference series		(2) Test series		Ratios (2): (1)	
Trial	Rat Number	Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcyst counts
	1	25	206	36	178		
	2	14	173	30	290		
	3	22	172	41	156		
1	4	40	161	43	126		
	5	26	208	46	214		
	6	36	183	52	124		
	7	25	123	32	242		
	8	.....	.....	50	162		
	Mean	26.9	175.14	41.3	186.5	1.535	1.065
	9	16	80	35	75		
	10	24	239	27	199		
	11	18	124	37	97		
2	12	15	139	44	114		
	13	18	110	32	109		
	14	28	153	35	87		
	15	22	228	32	155		
	Mean	20.1	153.29	34.6	119.43	1.721	0.779
W						1.628	
F							0.922

ASSAY OF WHOLE WHEAT

Whole wheat grains were purchased from a local dealer, picked over by hand to remove foreign grains and other materials, and finally ground in a coffee mill. The product was fed at the 30 per cent level. According to Morrison's (1936) tables, whole wheat contains about 13.5 per cent protein, considerable amounts of vitamins B and E, considerably less vitamin G, and lacks vitamins A and D. As in the case of the other test rations, the lacking vitamins were supplied in the cod liver oil. The experiment was conducted in two trials involving 14 rats in the reference series and 15 in the test series. The data are set forth in table 4.

The *W*-value of 1.628 shows that the wheat recipients made unusually good growth during the growth period of record, comparatively though not absolutely better than the series that was fed hulled oats.

The *F*-value of .922 indicates that the ration with wheat was approximately as stimulating to the reproduction of the coccidia as the ration with yeast. The two trials differ somewhat in respect to the ratios of the oöcyst counts for the two series; but both favor a rather high coccidibios content in wheat.

It is to be recalled that Becker and Morehouse (1936) demonstrated the coccidium-growth-promoting property of wheat germ. We intend later to investigate wheat bran, wheat flour middlings and white wheat flour for the same property.

ASSAY OF LINSEED MEAL

The linseed oil meal purchased from a local dealer was said to be of the "old process" kind and to contain about 35 per cent protein. It was ground to a fine powder in a coffe mill, and fed at the 10 per cent level. According to Morrison (1936), linseed meal is an excellent protein supplement for most farm animals except poultry, but its vitamin content is not well known except for an appreciable amount of vitamin E. The data are represented in table 5.

A *W*-value of .80 tells the story of the weight gain on the rations for the growth period of record. It is somewhat lower than might have been expected for such a short period, but it is likely that while elements in the ration other than linseed meal supplied adequate amounts of vitamins A and D, vitamins B and G were exceedingly limited even though the casein was unextracted.

The *F*-value of .94 indicates that despite the poor rat growth on the linseed meal diet, there was practically as much coccidium-stimulant conferred on the ration by 10 parts of linseed meal as by 4 parts of powdered yeast.

TABLE 5. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 10 per cent linseed oil meal diets*

Trial	Rat Number	(1) Reference series		(2) Test series		Ratios (2):(1)	
		Weight gain (gm.)	Oöcysts 10^4	Weight gain (gm.)	Oöcysts 10^4	Weight gains	Oöcyst counts
1	1	23	122	25	142		
	2	34	147	29	91		
	3	30	135	32	114		
	4	45	69	29	95		
	5	42	130	20	228		
	Mean	34.8	120.6	27	134.0	0.78	1.11
2	6	45	98	36	90		
	7	53	75	47	84		
	8	57	90	41	36		
	9	51	154	33	60		
	10	42	83	45	119		
	Mean	49.6	100.0	40.4	77.8	0.81	0.77
W						0.80	
F							0.94

TABLE 6. *Oöcyst counts and weight gains for rats on 4 per cent powdered yeast and 10 per cent Swift's meat and bone meal diets*

		(1) Reference series		(2) Test series		Ratios (2): (1)	
Trial	Rat Number	Weight gain (gm.)	Oöcysts 10^6	Weight gain (gm.)	Oöcysts 10^6	Weight gains	Oöcyst counts
	1	27	164	4	53		
	2	25	161	4	39		
1	3	14	336	3	81		
	4	23	125	4	122		
	5	14	166	—5	78		
	Mean	20.6	190.4	2	74.6	0.1	.392
	6	27	153	3	35		
	7	20	211	0	27		
2	8	16	148	3	65		
	9	11	202	2	53		
	10	17	210	3	33		
	Mean	18.2	184.8	2.2	42.6	0.12	.231
W						0.11	
F							.311

ASSAY OF MEAT AND BONE MEAL

Swift's meat and bone meal (or meat scrap) was the product employed for the test. It was said to contain about 50 per cent protein, and for this reason was fed at the 10 per cent level. The writers have no information as to its vitamin content, but like tankage it probably contains some vitamin G but no vitamin B. The weight gains and oöcyst counts are recorded in table 6.

Weight gains were exceedingly poor on the meat scrap ration, for the table shows a *W*-value of only 0.111.

The *F*-value of .311 is likewise exceedingly low. It requires no formal statistical analysis to determine that the differences between the two series are significant. Evidently, then, meat and bone meal favors but slightly both rat-growth and coccidium-growth in the host.

DISCUSSION

It has been brought out clearly in a number of papers by Becker and Morehouse (reviewed in 1937) that the quantity of oöcysts eliminated during coccidian infection of the white rat is susceptible to modification through diet, and that one ration may have a greater stimulating effect upon the parasite's reproduction in the host than another, even though the rations differ in but one important respect. As an example of the latter statement, rats which consumed only a basal diet made up to 10 per cent with Fleischmann's powdered yeast eliminated more than 3 times as

many oöcysts as rats which received the basal ration with 10 parts of powdered pork liver and 4 parts of rice polish in place of yeast. The present study was undertaken in order to determine whether basal rations that differed in respect to supplements other than yeast and liver and rice polish might likewise exert differential influences upon coccidian infection. Then there were other questions for which more nearly complete answers were sought: Is the growth of the host the factor which regulates the intensity of the infection? Is vitamin G the growth stimulant? Is the bulk of the ration an important factor? The procedure (described above) for attacking these problems was based upon comparing oöcyst counts and weight gains for animals on the test rations with the same for animals on the ration made up to 4 per cent with Fleischmann's powdered yeast.

Yellow corn meal, hulled oats, oat hulls, and wheat were used at the 30 per cent level with strikingly different results. The respective *F*-values were .474, .591, .494, and .922. Thus, the yellow corn meal and oat hull rations exerted only half as much growth stimulating effect upon the coccidium as did wheat, and hulled oats something less than two-thirds as much. For the 10 per cent linseed meal and meat and bone meal rations the *F*-values of .94 and .31 indicated vastly different effects. Several other interesting comparisons are as follows: the 10 per cent linseed meal diet gave about the same *F*-value as the 30 per cent wheat diet; 10 per cent meat and bone meal gave a smaller *F*-value than 30 per cent oat hulls; and all the vegetable products gave larger *F*-values than meat and bone meal, the only animal product tested.

In a previous paper Becker and Morehouse (1937) have given reasons for their view that the growth of the parasite is not simply a function of that of the host; or, that common factors favor the growth of both host and parasite. The present work adds more evidence for that conclusion; for, on the one hand, we find the linseed meal ration with a *W*-value of only .80 and an *F*-value of .94, and, on the other, the wheat ration with a *W*-value of twice as much (1.628) and an *F*-value of about the same (.922) as the linseed meal ration. Also, conversely, a comparison may be made between the yellow corn meal ration with an *F*-value of .474 and the oat hull ration with an *F*-value of about the same, or .494; but the former has a *W*-value of 1.092 and the latter only .688, or slightly over three-fifths as much. These observations, in addition to those previously published, seem to prove definitely that the factor, or set of factors, that favors the parasite's growth is different from that which is necessary for good host growth. At least it is not possible to avoid such a conclusion at the present time.

Is the coccidium-stimulant identical with vitamin G? Becker and Morehouse (1937) have already stated reasons for considering it to be different. The present findings are not so conclusive as the former, but are in harmony with them. The case of linseed meal may be used as an example. According to certain authors, it is known to contain rather considerable amounts of vitamin B, i. e., B_1. There is practically no information available regarding its vitamin G (or B_2) content. The limited growth we obtained with a basal ration made up to 15 per cent with unextracted commercial casein and to 10 per cent with linseed meal would indicate that vitamin G might have been the limiting factor. Nevertheless, the *F*-value for this diet was rather high (0.94).

One naturally ponders whether the bulk of the diet might not exert some mechanical effect upon the intestine that would affect the development of the coccidium. The experiments supply indirect evidence for the conclusion that the coccidium-growth effects observed are not to be accounted for on the basis of any such simple hypothesis. Referring again to Morrison's tables, corn has 2.2 per cent fiber; hulled oats, 1.9 per cent; whole oats, 10.6 per cent; wheat, 2.4 per cent; linseed meal, 8 per cent; and meat and bone scrap, 2.0 per cent. The amount of fiber in oat hulls was not shown, but it must be exceedingly high in view of the discrepancy between the percentages in hulled and whole oats. If the feeding stuffs are arranged according to fiber content, the following order is obtained: hulled oats, meat and bone meal, yellow corn meal, wheat, linseed meal, oat hulls. If now they are arranged according to *F*-values, they fall into the following order: meat and bone meal, yellow corn meal, oat hulls, hulled oats, wheat, linseed meal. In view of such a situation it is impossible to argue for or against either a hypothesis that fiber in the ration tends to provide conditions favorable for the development of the coccidium or one that fiber provides conditions that tend to restrict the parasite's activities.

All in all, these further studies tend to add evidence sustaining the hypothesis that there occurs in foods used by animals more or less of something of a chemical nature that stimulates coccidium-growth. This hypothetical principle was called coccidibios in a previous paper (1937). Further studies, however, will be necessary in order definitely to establish its existence and the nature of its action.

SUMMARY

A procedure for assaying feeding stuffs for their relative amounts of coccidium *(Eimeria nieschulzi)*-growth-promoting properties has been outlined. It is based upon a comparison of the numbers of the terminal stages of the protozoön discharged when the host (white rat) is consuming the tested material in a basal ration at the standard level (30 per cent for ordinary grains, etc., 10 per cent for concentrates) with the number discharged by the reference host on the basal ration made up to 4 per cent with Fleischmann's powdered yeast. It is a necessary condition of the experiment that the rats have not previously been infected and that the infective doses of the microörganism are the same in quantity and administered at the same time in test and reference series.

The ratios of the number of oöcysts from test animals to those from reference animals (*F*-values) for the rations made up with yellow corn meal, hulled oats, oat hulls, whole wheat, linseed meal, and meat and bone meal are, respectively, .474, .591, .494, .922, .94, and .311.

Attempts to correlate the *F*-values with weight gains made by the hosts during the first 16 or 17 days on the diets do not justify an assumption that the same qualities of the diet promote the growth of host and parasite. Neither is there a definite correlation between the bulkiness of the ration as measured by crude fiber and the development of the parasite as measured by oöcyst production.

The experiment further supports the earlier hypothesis that there occurs in feeding stuffs a coccidium-growth-promoting substance. In our earliest work it was considered to be vitamin G, but it is now considered

to be a distinct, though possibly related material. These conclusions apply to the rat infection.

LITERATURE CITED

BECKER, E. R., AND N. F. MOREHOUSE
1937. Modifying the quantitative character of coccidian infection through a dietary factor. Jour. Parasit., 23:153-162.

MORRISON, F. B.
1936. Feeds and feeding. 20th Ed. Ithaca, N. Y.

ROUDABUSH, R. L.
1937. The endogenous phases of the life cycles of *Eimeria nieschulzi, Eimeria separata,* and *Eimeria miyairii* coccidian parasites of the rat. Iowa State College Jour. Sci., 11:135-163.

USE OF A DISCRIMINANT FUNCTION FOR DIFFERENTIATING SOILS WITH DIFFERENT AZOTOBACTER POPULATIONS[1]

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After measuring several characteristics of each member of two or more groups, the investigator may wish to know if the groups differ significantly. The usual method is to test the significance of the difference between the group means, taking each character separately. But unfortunately, there is no way to combine the knowledge gained. The value of the information furnished by the several varieties may be different. Furthermore, correlation among the variates will make it inappropriate to treat the differences as independent.

Another method used is the Coefficient of Racial Likeness (6), which gives a single numerical measure of the whole system of differences. The Coefficient of Racial Likeness is made up of the sums of squares of the differences between the means of the variates in the two groups being compared, each squared difference divided by the corresponding variance. As pointed out by Karl Pearson, "The fundamental weakness of the Coefficient of Racial Likeness lies in the fact that it neglects the correlations between the characters dealt with."

Some recent articles (1, 2, 4, 7) present and illustrate a method for the differentiation of two or more groups which have been measured in several characters. This method has advantages over either of the methods just mentioned. The measured characters may or may not be correlated. They are combined to form a discriminant function which will give the maximum differences among the groups relative to the variance of the function within the groups. That is, a compound is chosen so that the "overlap" of the groups is a minimum. This method, as given by Professor Fisher, will be illustrated in the present article. A few modifications have been introduced in order to make available the methods of calculation described by Wallace and Snedecor (8).

A number of samples of Iowa soils were collected and examined for the presence of Azotobacter (5). One hundred of these samples were found to contain the organisms while 186 of them did not contain any. The pH, the available phosphate content and the total nitrogen content of the samples were determined. The data obtained required the calculation of the discriminant function in order to bring out the maximum difference between the two groups of soil samples. From the results obtained it will be possible (a) to determine whether these chemical soil measurements give significant information about the presence of Azotobacter and (b) to determine the relative value of these variates for such a discrimination.

Table 1 contains the data for the 100 samples which contained Azotobacter; table 2 the data for the 186 samples which did not. The pH of the individual samples ranges from 5.0 to 8.6, the amount of readily avail-

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able phosphates from 16 to 520 pounds per acre, and the total nitrogen content from 7 to 78 milligrams per 10 grams of soil. The mean value for each variate, for the samples in group I and group II, is given in table 3. The samples which contained Azotobacter have, on an average, a higher pH, more available phosphate and a larger total nitrogen content than the samples which did not contain the organisms. The mean differences, 1.408, 82.007, and 8.260, are given at the bottom of the table. The variates used:

X_1 = pH,

X_2 = the amount of readily available phosphate,

X_3 = the total nitrogen content,

are quite different in numerical size with correspondingly large differences in variance.

What weighted compound of the three variates will afford the maximum differentiation between the two groups of soil samples? Or, what coefficients of the linear function of the three variates,

TABLE 1. *pH(X_1), available phosphate content (X_2) and total nitrogen content (X_3) of 100 samples of Iowa soils which contained Azotobacter*

X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3
6.0	46	24	7.1	348	30	8.3	208	19	8.4	77	58
7.0	35	17	8.0	130	34	8.1	160	24	8.2	117	44
8.4	115	28	6.6	55	22	8.5	138	12	7.8	65	32
5.8	35	17	8.0	62	44	8.5	149	11	6.8	160	14
6.9	55	25	8.0	160	53	8.5	72	15	8.0	416	29
7.8	52	29	8.0	149	39	8.4	83	13	8.7	97	42
7.8	52	29	8.0	149	39	8.4	83	13	8.1	97	42
6.9	208	58	8.0	149	59	8.2	90	10	8.2	138	38
7.0	70	13	6.7	174	45	8.4	138	16	6.8	173	21
6.7	35	16	7.4	70	78	8.6	174	14	6.8	95	23
6.2	27	44	6.1	114	51	6.5	111	35	7.0	520	31
6.9	52	27	6.8	52	31	6.2	240	18	8.2	260	12
8.0	60	58	6.3	80	54	7.0	297	31	7.4	232	9
8.0	156	68	8.1	44	20	6.5	140	31	8.2	189	14
8.0	90	37	7.5	55	14	7.4	106	33	8.3	289	26
6.1	44	27	6.5	33	17	8.3	160	23	6.7	160	30
7.4	207	31	6.1	28	15	6.2	69	17	6.5	142	28
7.4	120	32	7.2	40	12	8.4	164	43	7.3	115	14
8.4	65	43	6.8	65	30	7.8	138	15	6.1	130	25
8.1	237	45	7.1	62	18	8.4	416	17	8.0	284	22
8.3	57	60	6.4	58	19	7.2	52	16	7.3	260	24
7.0	94	43	6.5	208	19	8.2	222	55	7.0	298	32
8.5	86	40	7.0	81	19	8.5	73	21	7.0	223	18
8.4	52	48	7.8	160	22	7.0	138	28	7.1	125	24
7.9	146	52	6.8	115	20	6.4	156	26	6.5	65	23

TABLE 2. *pH (X_1), available phosphate content (X_2) and total nitrogen content (X_3) of 186 samples of Iowa soils which contained no Azotobacter*

X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3	X_1	X_2	X_3
6.2	49	30	5.5	19	22	6.0	30	14	5.5	47	24	5.8	22	28	6.7	54	19	6.8	65	16
5.6	31	23	5.9	44	33	6.1	40	20	5.6	42	19	5.7	115	24	6.7	54	18	6.3	65	25
5.8	42	22	6.0	31	13	5.9	58	13	5.5	27	21	6.2	44	26	6.5	44	21	6.2	23	19
5.7	42	14	5.4	62	23	6.4	33	12	5.8	31	18	5.4	46	22	6.1	52	26	6.7	182	17
6.2	40	23	5.2	35	23	6.4	62	18	5.6	52	21	6.6	60	25	5.5	35	22	6.0	52	12
6.4	49	18	6.1	21	19	6.9	32	15	5.7	27	18	5.5	46	18	6.2	62	17	5.7	42	23
5.8	31	17	5.3	27	19	6.4	30	14	5.6	25	20	6.1	44	21	5.5	65	30	5.6	83	25
6.4	31	19	6.5	45	31	5.9	31	12	5.3	33	21	6.3	42	30	7.3	68	24	6.5	17	12
5.4	62	26	6.4	59	25	5.5	37	15	5.5	19	28	5.7	42	18	5.6	31	21	6.4	76	33
5.4	42	16	5.7	80	48	5.9	37	15	5.6	53	24	5.9	54	16	5.4	31	27	6.8	73	29
5.7	35	22	6.2	17	23	5.9	29	20	5.8	95	27	5.6	27	19	5.9	42	23	7.2	244	16
5.6	33	24	6.8	68	20	5.7	35	10	5.8	33	18	5.5	29	16	5.4	30	26	7.8	104	20
5.8	24	15	6.1	42	24	7.0	95	16	5.6	42	20	5.6	35	20	5.9	49	18	6.2	148	23
7.3	70	14	5.5	25	17	6.4	88	23	7.1	47	19	6.5	35	21	6.4	25	27	5.5	46	16
6.1	21	21	5.6	31	22	5.8	37	25	5.9	42	18	5.9	54	23	6.1	46	22	5.8	94	9
6.2	36	26	6.6	26	13	5.3	160	22	5.9	35	21	5.8	37	19	6.9	47	13	5.9	58	21
6.7	35	26	5.0	33	15	5.5	20	20	5.8	37	24	6.1	73	23	6.8	42	15	6.5	208	24
5.9	33	21	5.0	47	10	7.1	52	21	6.0	50	21	5.6	35	21	6.7	26	11	5.8	122	24
5.6	25	32	5.2	37	16	6.7	30	27	5.8	46	21	5.9	52	15	5.3	37	11	6.2	60	24
5.8	31	30	5.6	19	10	6.5	125	28	5.4	31	26	5.6	25	21	5.7	27	17	6.0	114	22
6.1	30	24	5.7	33	13	5.6	30	15	6.2	65	23	6.5	52	22	6.1	22	12	5.8	97	46
6.1	21	25	6.2	68	13	5.3	23	20	5.6	40	23	6.4	69	32	5.6	23	13	7.9	42	40
5.7	35	22	6.3	44	13	5.7	56	24	6.7	31	19	7.2	88	21	5.8	31	17	6.0	94	27
5.8	37	24	6.1	42	16	5.7	26	21	6.0	32	23	6.8	42	22	5.6	21	17	6.1	149	16
5.8	28	19	6.3	44	17	5.8	66	28	6.0	70	25	6.6	77	25	6.0	112	53			
5.7	34	20	5.9	35	24	5.6	35	18	5.9	33	12	6.3	57	27	6.5	189	22			
5.8	16	19	7.4	105	34	5.6	58	21	5.8	46	20	6.2	52	18	5.8	42	29			

$$X = L_1 \frac{\bar{x}_1}{\sqrt{\Sigma x_1^2}} + L_2 \frac{\bar{x}_2}{\sqrt{\Sigma x_2^2}} + L_3 \frac{\bar{x}_3}{\sqrt{\Sigma x_3^2}},$$

will maximize the ratio of the difference between the means of the groups to the standard deviation within the groups? X is the weighted compound of the measurements of pH, available phosphate and total nitrogen. The quantities L_1, L_2 and L_3 are the coefficients by which the respective measurements (each divided by the square root of the sum of squares *within groups*) of any individual soil samples should be multiplied in order to form its compound measurement X.

The difference between the means of X in the two groups is:

$$D = L_1 \frac{d_1}{\sqrt{\Sigma x_1^2}} + L_2 \frac{d_2}{\sqrt{\Sigma x_2^2}} + L_3 \frac{d_3}{\sqrt{\Sigma x_3^2}},$$

where d_1, d_2 and d_3 are the mean differences between the three variates in the two groups. The problem is then to find the values of L_1, L_2 and L_3 such that D is a maximum. The method involves the solution of a set of normal equations similar to those leading to multiple regression. Since there are two groups of observations, some of the calculations are like those which have become familiar in analysis of variance. The new features are to be described in some detail.

TABLE 3. *Number of samples, sums, means and mean differences for pH, available phosphate content and total nitrogen content*

Group		pH X_1	Phosphate X_2	Nitrogen X_3
I. With Azotobacter	Number	100	100	100
	Sum	742.3	13312	2940
	Mean	7.423	133.120	29.400
II. Without Azotobacter	Number	186	186	186
	Sum	1118.7	9507	3932
	Mean	6.015	51.113	21.140
Mean difference		1.408	82.007	8.260

In table 4 are recorded the computations leading to the pooled sums of squares and products within the two groups of soil measurements. In the line of totals, the entries are the sums of squares and products of the entire 286 observations in tables 1 and 2, no distinction being made as to group. In the lines for groups are put down the sums of squares and products of the group sums in table 3, calculated in the manner characteristic of analysis of variance. As examples, the entry for column X_1 in row X_1 of table 4 is,

$$\frac{(742.3)^2}{100} + \frac{(1{,}118.7)^2}{186} = 12{,}238.5321,$$

and for column X_2, row X_1,

TABLE 4. *Calculation of the correlation coefficients and the standard deviations within the groups*

		pH X_1	Phosphate X_2	Nitrogen X_3
X_1	Total	12,349.62	158,287.7	45,671.1
	Groups	12,238.5321	155,994.9808	45,472.6974
	Within groups	$\Sigma x_1^2 =$ 111.0879	$\Sigma x_1 x_2 =$ 2,292.7192	$\Sigma x_1 x_3 =$ 198.4026
		$\sqrt{\Sigma x_1^2} =$ 10.5398	$\sqrt{\Sigma x_1^2}\sqrt{\Sigma x_2^2} =$ 10,762.9845	$\sqrt{\Sigma x_1^2}\sqrt{\Sigma x_3^2} =$ 1,807.8866
		$s_{x1} =$ 0.625422	$r_{x1x2} =$ .213019	$r_{x1x3} =$ .109743
X_2		Total 3,300,823.		597,415.
		Groups 2,258,023.8110		592,348.7355
		Within groups $\Sigma x_2^2 =$ 1,042,799.1890		$\Sigma x_2 x_3 =$ 5,066.2645
		$\sqrt{\Sigma x_2^2} =$ 1,021.1754		$\sqrt{\Sigma x_2^2}\sqrt{\Sigma x_3^2} =$ 175,161.7058
		$s_{x2} =$ 60.595614		$r_{x2x3} =$ .028823
			Total 198,980.	
			Groups 169,557.6344	
			Within groups $\Sigma x_3^2 =$ 29,422.3655	
			$\sqrt{\Sigma x_3^2} =$ 171.5295	
			$s_{x3} =$ 10.178404	

$$\frac{(742.3)\ (13{,}312)}{100} + \frac{(1{,}118.7)\ (9507)}{186} = 155{,}994.9808.$$

The differences in the third line are the sums of squares and products of deviations from means within the groups.

The calculation of the standard deviations and the correlation coefficients now proceeds in the usual manner (8, table 7a, page 32). As examples,

$$s_{x1} = \frac{\sqrt{111.0879}}{\sqrt{284}} = \frac{10.5398}{\sqrt{284}} = 0.625422,$$

$$r_{x1x2} = \frac{2{,}292.7192}{(10.5398)\ (1{,}021.1754)} = 0.213019.$$

The degrees of freedom used, 284, are those within the two groups, $(100 - 1) + (186 - 1)$.

It may be observed that the pooled standard deviations of these variates are very different, and that there is little correlation between the variates within the two groups. It is usually of interest to observe these statistics, and it takes little extra time to compute them. In addition, it has been found convenient to use the correlation coefficients in the solution of the normal equations which follow.

The correlation coefficients from table 4 are carried into table 5 where they are used to solve the linear function, X, which best discriminates the two groups of soils. The coefficients (L_1, L_2 and L_3) required are proportional to the solutions of the equations,

$$r_{11}L_1 + r_{12}L_2 + r_{13}L_3 = 1, \quad 0, \quad 0,$$

$$r_{12}L_1 + r_{22}L_2 + r_{23}L_3 = 0, \quad 1, \quad 0,$$

$$r_{13}L_1 + r_{23}L_2 + r_{33}L_3 = 0, \quad 0, \quad 1.$$

Each expression, in turn, is set equal to 1 with the other expressions equal to 0.

Table 5 is worked in a manner similar to table 8 (page 36) in Wallace and Snedecor (8) except for the three back solutions. The k values obtained constitute the matrix in table 6. They will be used below to calculate the desired L coefficients.

Going back, now, to the mean differences given at the bottom of table 3,

$$d_1 = 1.408,$$

$$d_2 = 82.007,$$

$$d_3 = 8.260,$$

each difference is divided by the square root of its sum of squares within the groups, thus,

TABLE 5. *Solution of equations, with each equation, in turn, set equal to 1 with the other equations equal to 0*

	pH	Phosphate	Nitrogen				Sum
	1.000000	.213019	.109743	1.000000			2.322762
	−1.000000	− .213019	− .109743	−1.000000			−2.322762
		1.000000	.028923	.0	1.000000		2.241942
		− .045377	− .023377	− .213019	.0		− .494792
		.954623	.005546	− .213019	1.000000		1.747150
		−1.000000	− .005810	+ .223145	−1.047534		−1.830199
			1.000000	.0	.0	1.000000	2.138666
			− .012044	− .109743	.0	.0	− .254907
			− .000032	.001238	− .005810	.0	− .010151
			.987924	− .108505	− .005810	1.000000	1.873608
			−1.000000	+ .109831	+ .005881	−1.012224	−1.896510
		k_{13} =	− .109831	− .109831			
	k_{12} =	− .222506	+ .000638	− .223144			
k_{11} =	1.059451	+ .047398	+ .012053	+1.000000			
		k_{23} =	− .005881	− .005881			
	k_{22} =	1.047568	+ .000034	+1.047534			
k_{21} =	− .222507	− .223152	+ .000645	.0			
		k_{33} =	1.012224	1.012224			
	k_{32} =	− .005881	− .005881	.0			
k_{31} =	− .109831	+ .001253	− .111084	.0			

$$\frac{d_1}{\sqrt{\Sigma x_1^2}} = \frac{1.408}{10.5398} = .133589,$$

$$\frac{d_2}{\sqrt{\Sigma x_2^2}} = \frac{82.007}{1021.1754} = .080306,$$

$$\frac{d_3}{\sqrt{\Sigma x_3^2}} = \frac{8.260}{171.5295} = .048155.$$

Multiplying the columns of the matrix in table 6 by these adjusted differences gives the values of the coefficients L_1, L_2 and L_3:

$L_1 = (1.059451)(.133589) + (-.222506)(.080306) + (-.109831)(.048155) = .118374,$

$L_2 = (-.222507)(.133589) + (1.047568)(.080306) + (-.005881)(.048155) = .054118,$

$L_3 = (-.109831)(.133589) + (-.005881)(.080306) + (1.012224)(.048155) = .033599.$

TABLE 6. *Matrix of multipliers reciprocal to the correlations within groups*

	pH	Phosphate	Nitrogen
pH	k_{11} 1.059451	k_{12} −0.222506	k_{13} −0.109831
Phosphate	k_{21} −0.222507	k_{22} 1.047568	k_{23} −0.005881
Nitrogen	k_{31} −0.109831	k_{32} −0.005881	k_{33} 1.012224

For the linear function, X, of the three variates, the difference between the means of X in the two groups is, as noted above,

$$D = L_1 \frac{d_1}{\sqrt{\Sigma x_1^2}} + L_2 \frac{d_2}{\sqrt{\Sigma x_2^2}} + L_3 \frac{d_3}{\sqrt{\Sigma x_3^2}},$$

hence, $D = (.118374)(.133589) + (.054118)(.080306) + (.033599)(.048153) = .021777.$

This value of D is the greatest difference that can be obtained from a linear compound of the measured characters of the two groups of soils.

In order to test the significance of the difference just computed, the sum of squares of the compound X is now analyzed into two parts, within the groups and between the groups. The *between groups* sum of squares is:

$$\frac{n_1 n_2}{n_1 + n_2} D^2 = \frac{(100)(186)}{100 + 186} (.021777)^2 = (65.035)(.00047424) = .030842.$$

This value is entered in table 7. The sum of squares *within groups* is the value of D, 0.021777. In determining the degrees of freedom between groups, note that in addition to the specific mean difference, two adjustable ratios have been used, making the three degrees of freedom. The analysis of variance is completed in table 7. The difference between the group means is highly significant, showing that the pH, available phosphate and total nitrogen content of the soils tested give significant information about the presence of Azotobacter.

TABLE 7. *Analysis of variance of the crude compound X between and within groups*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Between groups	3	.030842	.01028
Within groups	282	.021777	.00007722
Total	285		

The relative value of these variates for discriminating between the groups is apparently indicated by the values of the coefficients,

$$L_1 = .118374,$$
$$L_2 = .054118,$$
$$L_3 = .033599.$$

It may be concluded from these results, therefore, that the pH, the content of available phosphate and of total nitrogen serve to significantly distinguish the samples of Iowa soils which contained Azotobacter from those which contained none of the bacteria. In addition, the results indicate that the presence of Azotobacter in Iowa soils may be most closely associated with the pH, closely associated with the available phosphate content of the soil and least associated with its total nitrogen content.

LITERATURE CITED

1. Barnard, M. M.
1935. The secular variations of skull characters in four series of Egyptian skulls. Ann. Eugen., 6:352-371.

2. Fisher, R. A.
1936. The use of multiple measurements in taxonomic problems. Ann. Eugen., 7:179-188.

3. ———
1934. Statistical methods for research workers. Ed. 5, Edinburgh, Oliver and Boyd.

4. Martin, E. A.
1936. A study of an Egyptian series of mandibles, with special reference to mathematical methods of sexing. Biometrika, 28:149-178.

5. Martin, W. P., R. H. Walker and P. E. Brown
1937. The occurrence of Azotobacter in Iowa soils and factors affecting their distribution. Iowa Agr. Exp. Sta. Res. Bul. 217.

6. PEARSON, K.
1926. On the coefficient of racial likeness. Biometrika, 18:105-117.

7. SMITH, H. F.
1936. A discriminant function for plant selection. Ann. Eugen. 7:240-250.

8. WALLACE, H. A., AND G. W. SNEDECOR
1931. Correlation and machine calculation. Iowa State College Off. Pub., 30, No. 4, Revised edition.

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